EXHIBIT 6

US005843660A

United States Patent [19]

Schumm et al.

[11] **Patent Number: 5,843,660**

[45] **Date of Patent: Dec. 1, 1998**

[54] **MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI**

[75] Inventors: **James W. Schumm**, Madison; **Katherine A. Micka**, Oregon; **Dawn R. Rabbach**, DeForest, all of Wis.

[73] Assignee: **Promega Corporation**, Madison, Wis.

[21] Appl. No.: **632,575**

[22] Filed: **Apr. 15, 1996**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 316,544, Sep. 30, 1994.

[51] **Int. Cl.**[6] **C12Q 1/68**; C12P 19/34; C07H 21/04

[52] **U.S. Cl.** **435/6**; 435/91.2; 536/24.33; 935/77; 935/78

[58] **Field of Search** 435/5, 6, 91.1, 435/91.2; 536/23.1, 24.3, 24.33

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Primary Examiner—Stephanie W. Zitomer
Attorney, Agent, or Firm—Grady J. Frenchick; Stroud, Stroud, Willink, Thompson & Howard; Karen B. King

[57] **ABSTRACT**

The present invention is directed to the simultaneous amplification of multiple distinct genetic loci using PCR or other amplification systems to determine in one reaction the alleles of each of the loci contained within the multiplex.

43 Claims, 19 Drawing Sheets

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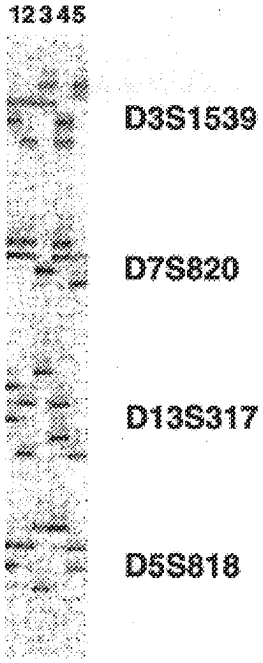


FIGURE 1

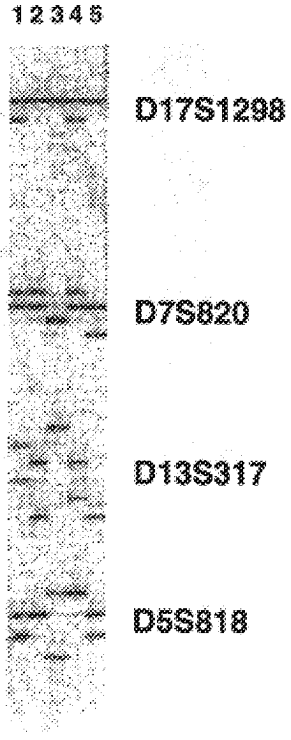


FIGURE 2

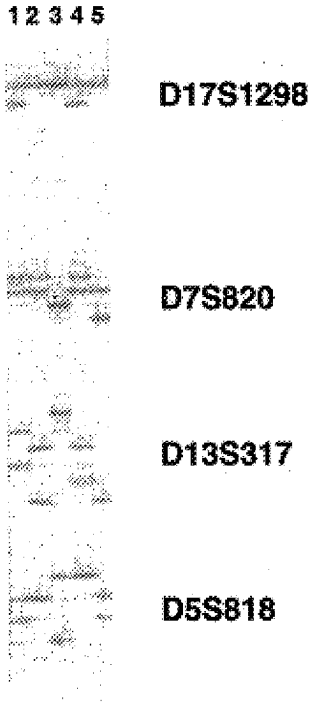
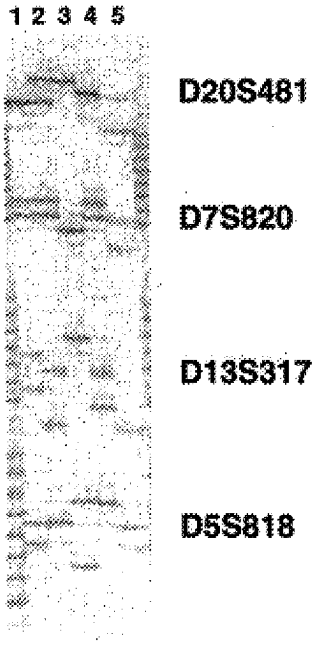


FIGURE 3



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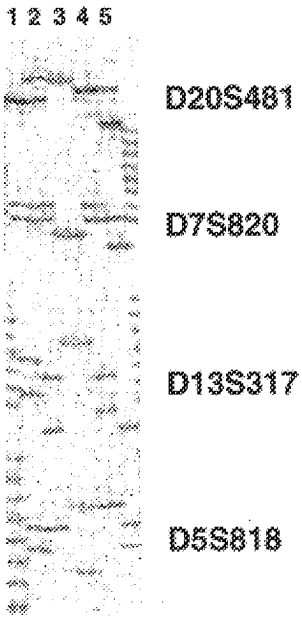
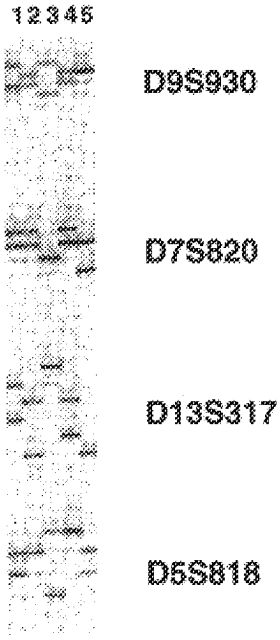


FIGURE 5



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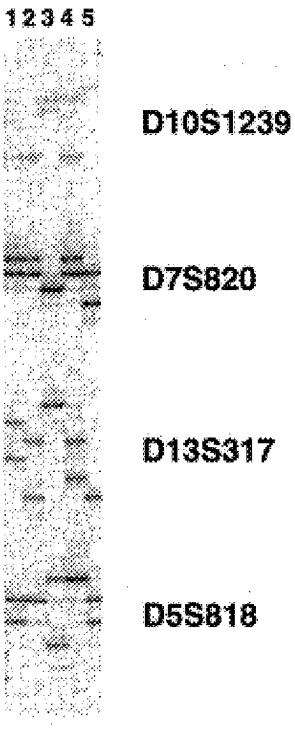

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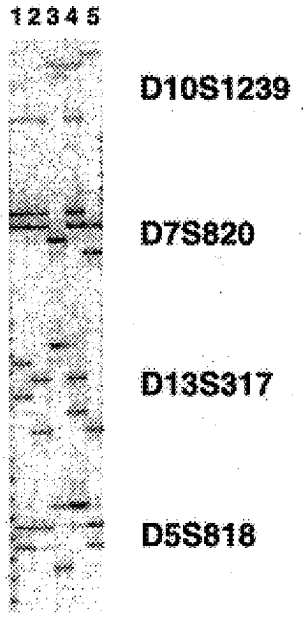

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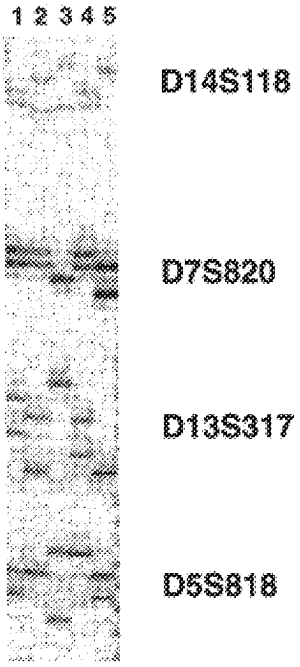

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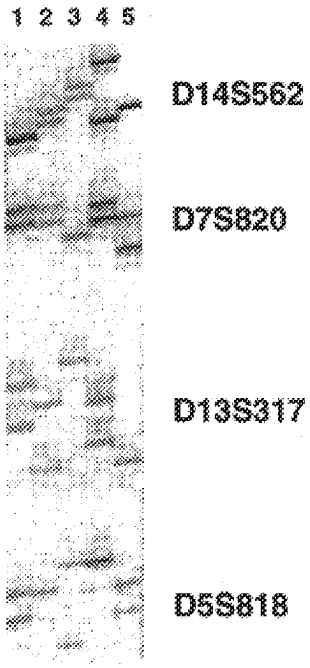

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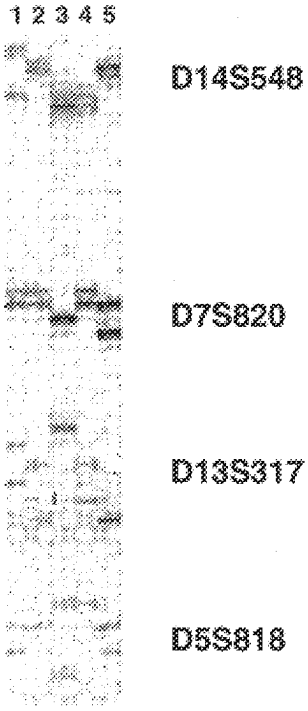
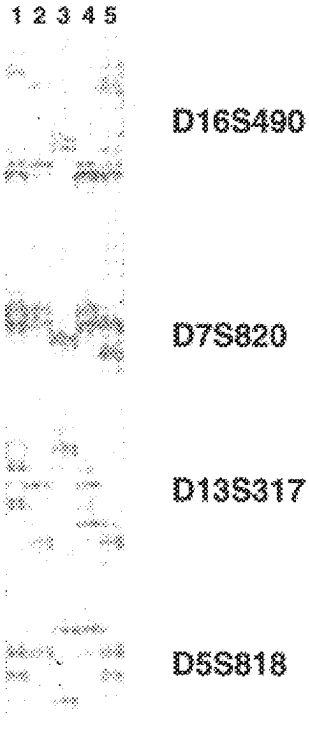


FIGURE 11



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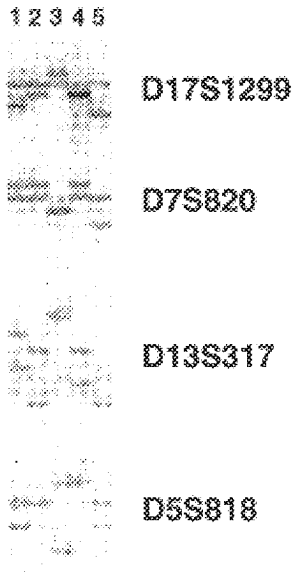

FIGURE 14

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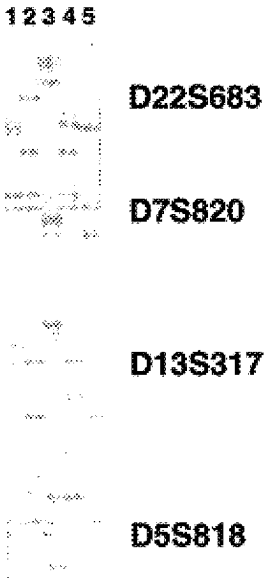

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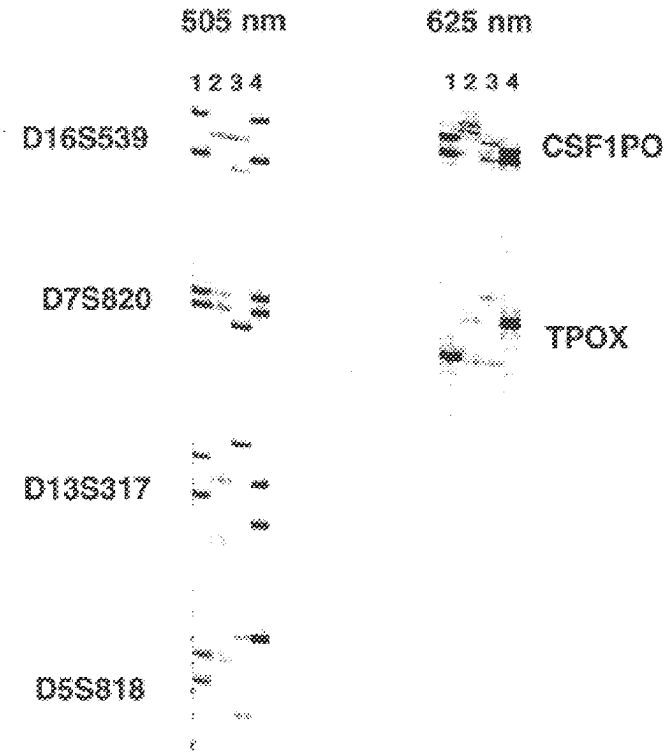

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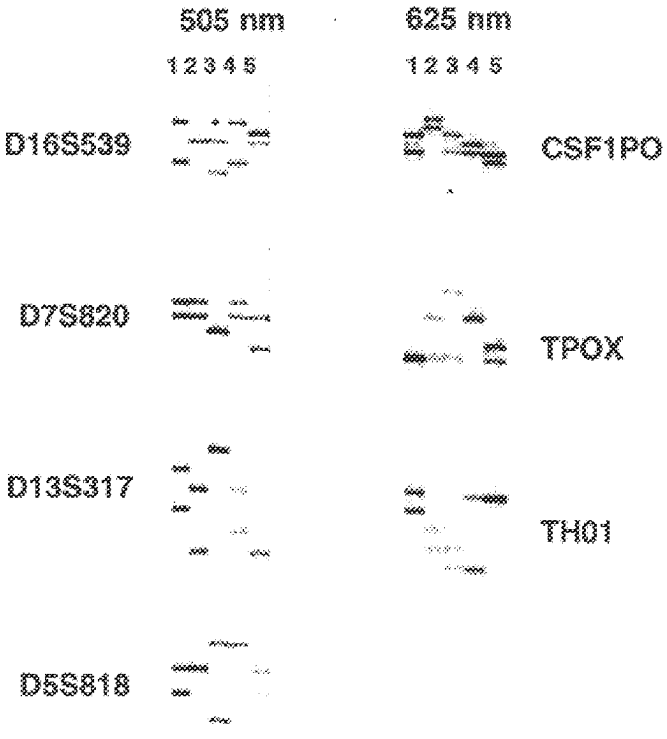

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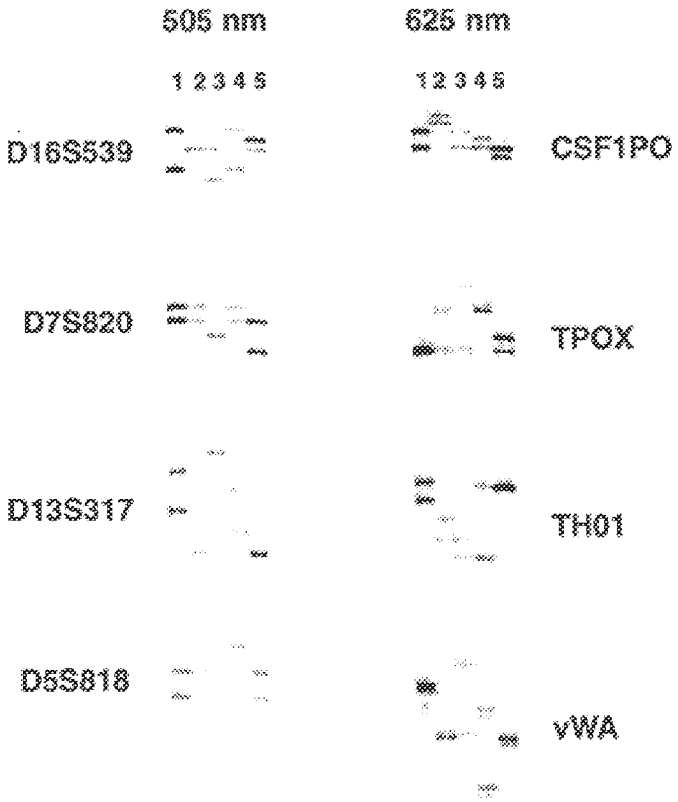
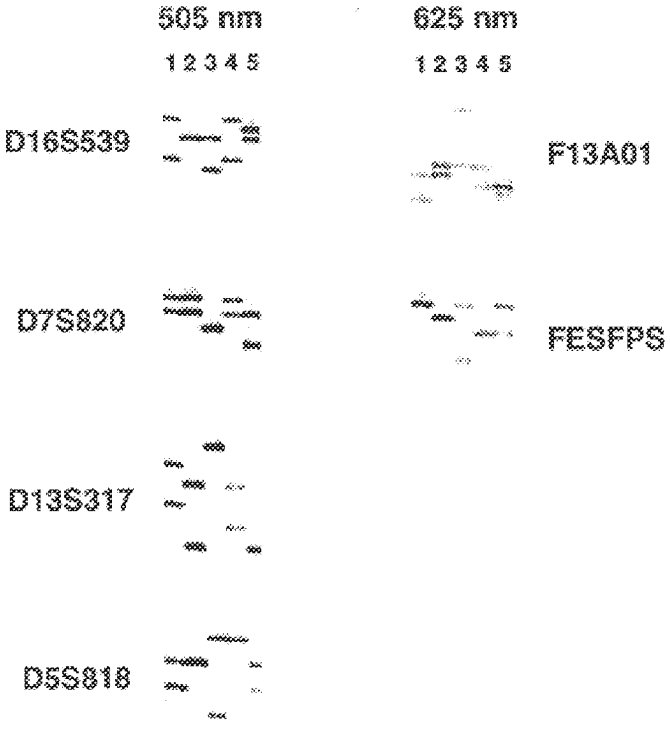


FIGURE 19



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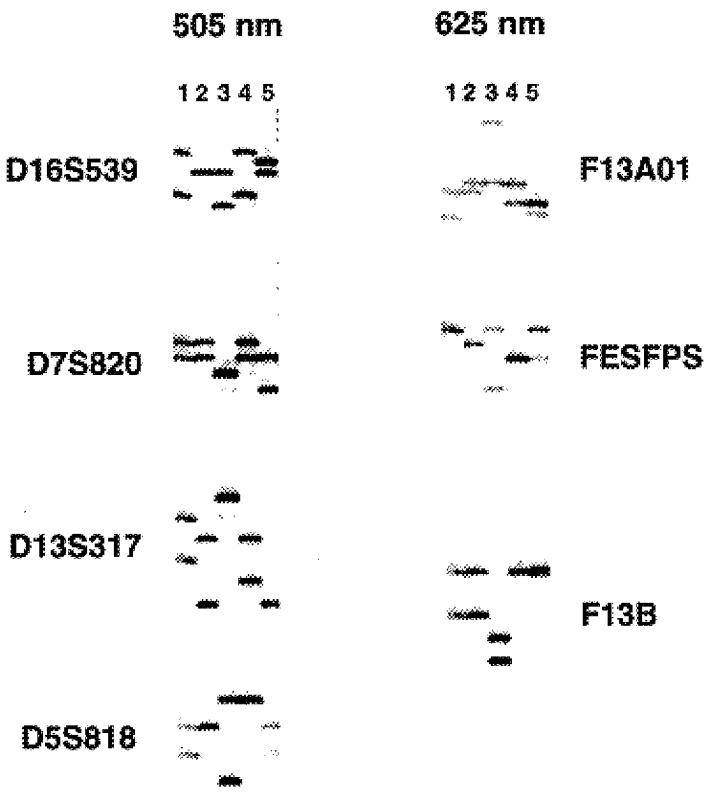

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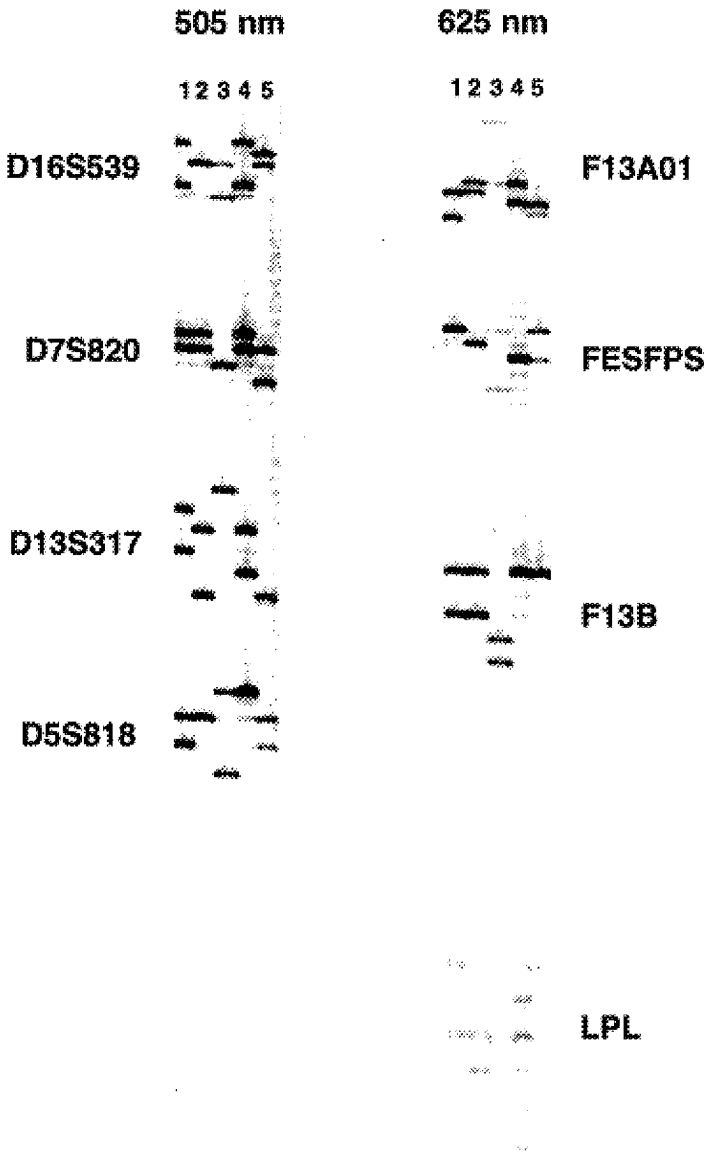

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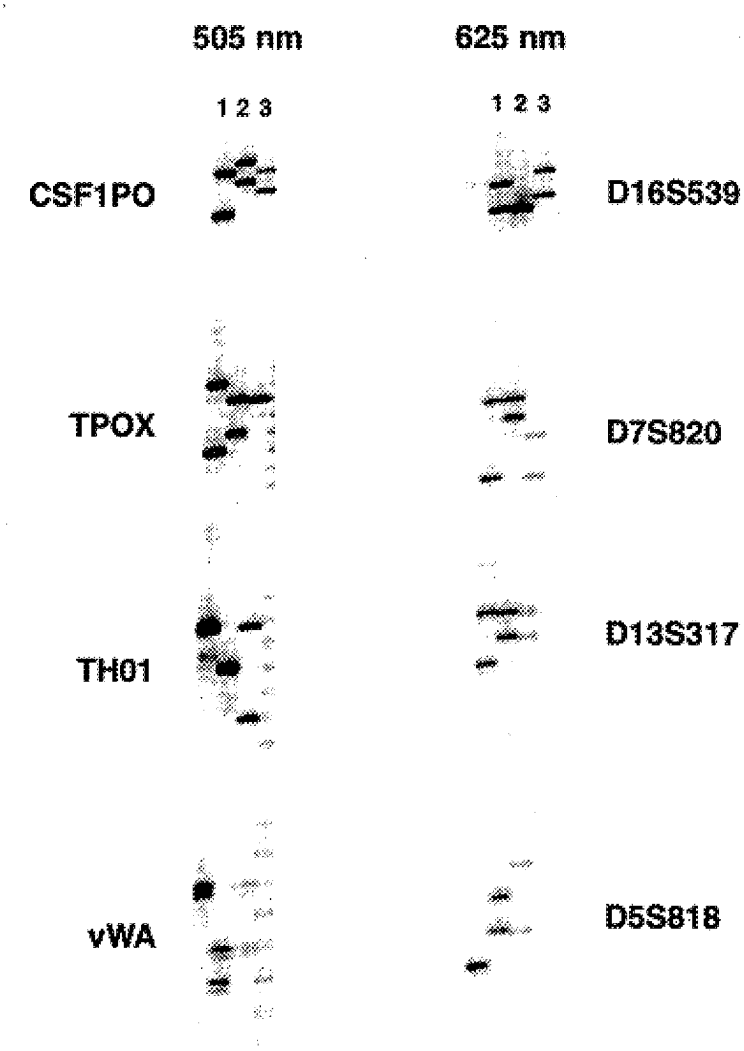

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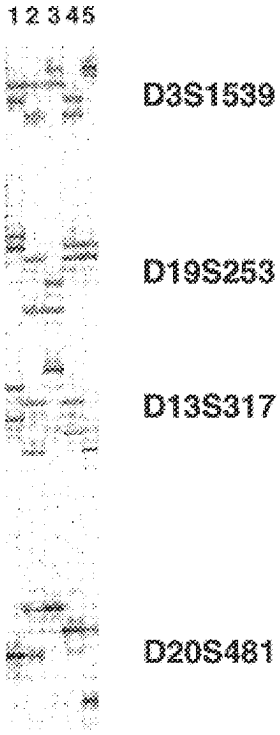

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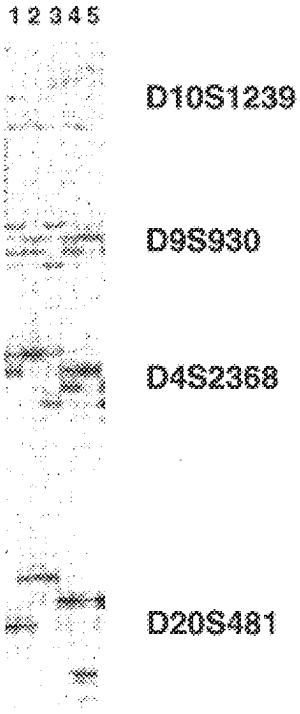

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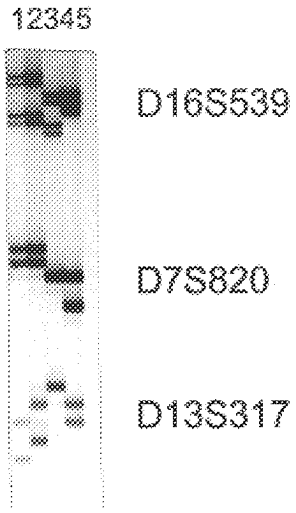
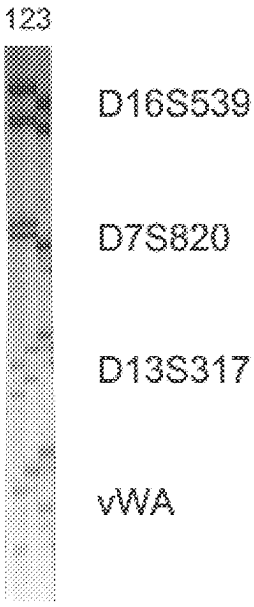


FIGURE 26



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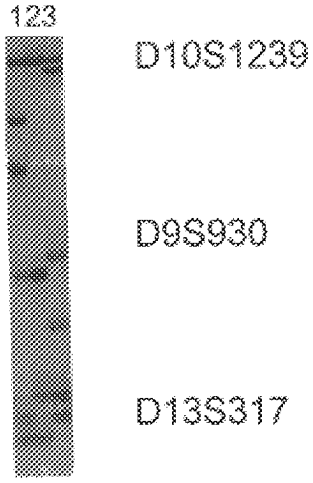

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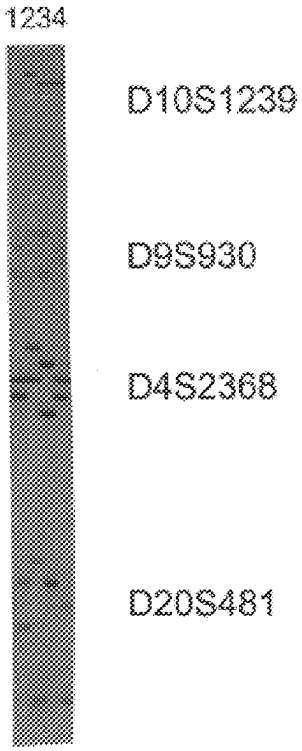

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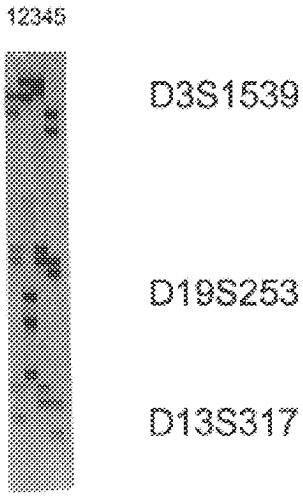

FIGURE 31

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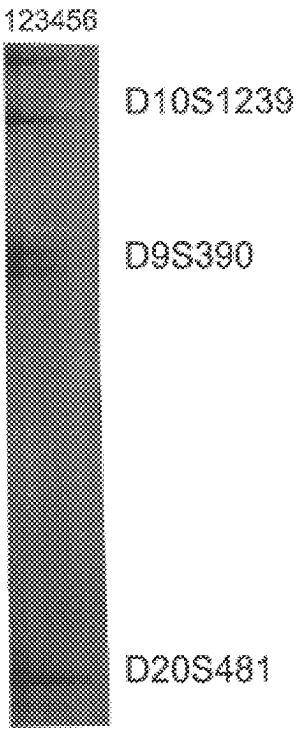

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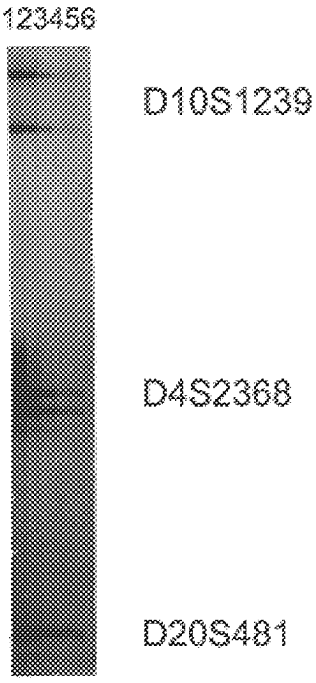

FIGURE 35

MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI

This application is a continuation-in-part of U.S. patent application Ser. No. 08/316,544, filed Sep. 30, 1994. The entire disclosure of that parent application is incorporated by reference herein.

FIELD OF THE INVENTION

The present invention is generally directed to the detection of genetic markers in a genomic system. The present invention is more specifically directed to the simultaneous amplification of multiple distinct polymorphic genetic loci using the polymerase chain reaction or other amplification systems to determine in one reaction the alleles of each locus contained within the multiplex system.

BACKGROUND OF THE INVENTION

In recent years, the discovery and development of polymorphic short tandem repeats (STRs) as genetic markers has stimulated progress in the development of linkage maps, the identification and characterization of diseased genes, and the simplification and precision of DNA typing.

Many loci, at least in the human genome, contain polymorphic STR regions (Adamson, D., et al. (1995) "A collection of ordered tetranucleotide-repeat markers from the human genome," *Am. J. Hum. Genet.* 57: 619–628; Murray, J. C., et al. (1994) "A comprehensive human linkage map with centimorgan density," *Science* 265: 2049–2054; Hudson, T. J., Engelstein, M., Lee, M. K., Ho, E. C., Rubenfield, M. J., Adams, C. P., Housman, D. E., and Dracopoli, N. C. (1992) "Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms," *Genomics* 13: 622–629). STR loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length. It is estimated that there are 2,000,000 expected trimeric and tetrameric STRs present as frequently as once every 15 kilobases (kb) in the human genome (Edwards et al. (1991) "DNA typing and genetic mapping with trimeric and tetrameric tandem repeats." *Am. J. Hum. Genet.* 49: 746–756; Beckman, J. S., and Weber, J. L. (1992) "Survey of human and rat microsatellites," *Genomics* 12: 627–631). Nearly half of the STR loci studied by Edwards et al. (1991) are polymorphic, which provides a rich source of genetic markers.

Variation in the number of short tandem repeat units at a particular locus causes the length of the DNA at that locus to vary from allele to allele and from individual to individual. Such length polymorphism is reminiscent of variable number of tandem repeats (VNTR) loci (Nakamura, Y., et al. (1987) "Variable number of tandem repeat (VNTR) markers for human gene mapping," *Science* 235: 1616–1622) and minisatellite loci (Jeffreys, A. J., et al. (1985) "Hypervariable 'minisatellite' regions in human DNA," *Nature* 314: 67–73), both of which contain considerably longer repeat units than STR loci. Such length polymorphism is also reminiscent of the dinucleotide repeat form of microsatellite loci (Litt, M. and Luty, J. A. (1989) "A hypervariable microsatellite revealed by in-vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene," *Am. J. Hum. Genet.* 44: 397–401, Tautz, D., et al. (1986) "Cryptic simplicity in DNA is a major source of genetic variation," *Nature* 322: 652–656, Weber, J. L. and May, P. E. (1989) "Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction," *Am. J. Hum. Genet.* 44: 388–396; Beckmann and Weber, (1992)), a form of microsatellite loci with shorter repeat units than STR loci.

Polymorphic STR loci are extremely useful markers for human identification, paternity testing and genetic mapping. STR loci may be amplified via the polymerase chain reaction (PCR) by employing specific primer sequences identified in the regions flanking the tandem repeat.

Alleles of these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another following electrophoretic separation by any suitable detection method including radioactivity, fluorescence, silver stain, and color.

To minimize labor, materials and analysis time, it is desirable to analyze multiple loci and/or more samples simultaneously. One approach for reaching this goal involves amplification of multiple loci simultaneously in a single reaction. Such "multiplex" amplifications, as they are called, have been described extensively in the literature. Multiplex amplification sets have been extensively developed for analysis of genes related to human genetic diseases such as Duchenne Muscular Dystrophy (Chamberlain, J. S., et al. (1988) "Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification," *Nucleic Acid Res.* 16: 11141–11156; Chamberlain, J. S., et al. (1989), "Multiple PCR for the diagnosis of Duchenne muscular dystrophy," In *PCR Protocols, A Guide to Methods and Application* (ed. Gelfand, D. H., et al.) pp. 272–281. Academic Press, San Diego, Calif.; Beggs, A. H., et al. (1990) "Detection of 98% DMD/BMD gene deletions by PCR," *Hum. Genet.* 86: 45–48; Clemens, P. R., et al. (1991). "Carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophy families, using dinucleotide repeat polymorphisms," *Am J. Hum. Genet.* 49: 951–960; Schwartz, J. S., et al. (1992) "Fluorescent multiple linkage analysis and carrier detection for Duchenne/Becker's muscular dystrophy," *Am J. Hum. Genet.* 51: 721–729; Covone, A. E., et al. (1992) "Screening Duchenne and Becker muscular dystrophy patients for deletions in 30 exons of the dystrophin gene by three-multiplex PCR," *Am. J. Hum. Genet.* 51: 675–677), Lesch-Nyhan Syndrome (Gibbs, R. A., et al. (1990) "Multiple DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families," *Genomics* 7: 235–244), Cystic Fibrosis (Estivill, X., et al. (1991) "Prenatal diagnosis of cystic fibrosis by multiplex PCR of mutation and microsatellite alleles," *Lancet* 338: 458; Fortina, P., et al. (1992) "Non-radioactive detection of the most common mutations in the cystic fibrosis transmembrane conductance regulator gene by multiplex polymerase chain reaction," *Hum. Genet.* 90: 375–378; Ferrie, R. M., et al. (1992) "Development, multiplexing, and application of ARMS tests for common mutations in the CFTR gene," *Am. J. Hum. Genet.* 51: 251–262; Morral, N. and Estivill, X. (1992) "Multiplex PCR amplification of three microsatellites within the CFTR gene," *Genomics* 51: 1362–1364), and Retinoblasma (Lohmann, D., et al. (1992) "Detection of small RB1 gene deletions in retinoblastoma by multiplex PCR and high-resolution gel electrophoresis," *Hum. Genet.* 89: 49–53). Multiplex amplification of polymorphic microsatellite markers (Clemens et al. (1991); Schwartz et al. (1992); Huang, T. H.-M., et al. (1992) "Genetic mapping of four dinucleotide repeat loci DXS435, DXS45, DXS454, DXS424, on the X chromosome using the multiplex polymerase chain reaction," *Genomics* 13: 375–380) and even STR markers (Edwards, A., et al. (1992) "Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups," *Genomics* 12: 241–253; Kimpton, C. P., et al.

(1993) "Automated DNA profiling employing multiplex amplification of short tandem repeat loci," *PCR Methods and Applications* 3: 13–22; Hammond, H. A., et al. (1994) "Evaluation of 13 STR loci for use in personal identification applications," *Am. J. Hum. Genet.* 55: 175–189; Schumm, J. W. et al. (1994) "Development of nonisotopic multiplex amplification sets for analysis of polymorphic STR loci," in "The Fourth International Symposium on Human Identification 1993," pp. 177–187; Oldroyd, N. J., et al. (1995) "A highly discriminating octoplex short tandem repeat polymerase chain reaction system suitable for human individual identification," *Electrophoresis* 16: 334–337) have been described.

These amplified products are generally separated by one of several methods of electrophoresis known to those skilled in the art. Several well-known methods of detection of the amplified products have also been described. While ethidium bromide staining or silver staining of amplified fragments is employed in some cases, in others it is preferred to use methods which label only one of the two strands of the amplified material. Examples of this include radioactive or fluorescent labeling of one of the two primers prior to the amplification of a locus. One of the more sophisticated approaches to detection is the use of different fluorescent labels to allow detection of amplified materials representing different loci, but existing in the same space following electrophoresis. The products of the different loci are differentiated with the use of filters or other discriminating detectors, which allow visualization of one fluorescent label at a time.

Reference is made to International Publications WO 93/18177 and WO 93/18178 to Fortina et al., which are directed to methods and kits for diagnosing diseases such as Cystic Fibrosis and β-thalassemia, respectively, using an allele-specific multiplex polymerase chain reaction system. According to Fortina et al., multiplex PCR has also been used for simultaneous amplification of multiple target sequences, permitting mutant allele scanning using two lanes of an agarose gel.

Ballabio, A. et al. (1991) "PCR Tests for Cystic Fibrosis Deletion," *Nature,* 343: 220, disclose a single-tube, multiplex allele-specific PCR test using two different dye-tagged fluorescent primers for detection of the F508 cystic fibrosis mutation.

While there are multiplex amplification procedures for specific loci, the use of multiplex amplification procedures is greatly desired for the detection of alleles in other types of loci such as specific STR loci. It is also desirable to identify primers which make multiplex amplification of such loci possible.

SUMMARY OF THE INVENTION

It is, therefore, an object of the present invention to provide a method and materials for the simultaneous amplification of multiple distinct polymorphic short tandem repeat (STR) loci using PCR or other amplification systems to determine, in one reaction, the alleles of each locus contained within the multiplex. Multiplex analysis of the sets of specific STR loci disclosed herein have not been previously described in the prior art. There has also not been any previous description of the sequences for many of the primers disclosed herein below, all of which are shown to be useful for multiplex amplification of such STR loci.

It is also an object of the present invention to provide a method, a kit, and primers specific for multiplex amplifications comprising specified loci.

These and other objects are addressed by the present invention which is directed to a method and materials for simultaneously analyzing or determining the alleles present at each individual locus of each multiplex. In general, the method of this invention comprises the steps of (a) obtaining at least one DNA sample to be analyzed, wherein the DNA sample has at least two loci which can be amplified together; (b) amplifying the STR sequences in the DNA sample; and (c) detecting the amplified materials in a fashion which reveals the polymorphic nature of the systems employed.

More specifically, the method of this invention is a method of simultaneously determining the alleles present in at least three tandem repeat loci from one or more DNA samples, such method comprising the steps of:

(a) obtaining at least one DNA sample to be analyzed, wherein the DNA sample has a set of at least three loci which can be amplified together, wherein the set of loci is selected from a specific group or sets of loci disclosed herein;

(b) co-amplifying the set of loci in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(c) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

In one embodiment of this invention, three STR loci are amplified together, and the set of three co-amplified loci is selected from the group of sets consisting of:

D3S1539, D19S253, D13S317;

D10S1239, D9S930, D20S481;

D10S1239, D4S2368, D20S481;

D10S1239, D9S930, D4S2368;

D16S539, D7S820, D13S317; and

D10S1239, D9S930, D13S317.

In a more preferred embodiment of the method of this invention, the DNA sample has at least four STR loci which can be amplified together, and the set of co-amplified loci is selected from the group of loci consisting of:

D3S1539, D4S2368, D5S818, D7S820, D9S930, D10S1239,

D13S317, D14S118, D14S548, D14S562, D16S490, D16S539,

D16S753, D17S1298, D17S1299, D19S253, D20S481, D22S683,

HUMCSF1PO, HUMTPOX, HUMTH01, HUMFESFPS, HUMF13A01,

HUMBFXIII, HUMLIPOL, HUMvWFA31.

The set of at least four STR loci amplified together is more preferably a set selected from the sets of four loci comprising:

D3S1539, D7S820, D13S317, D5S818;

D17S1298, D7S820, D13S317, D5S818;

D20S481, D7S820, D13S317, D5S818;

D9S930, D7S820, D13S317, D5S818;

D10S1239, D7S820, D13S317, D5S818;

D14S118, D7S820, D13S317, D5S818;

D14S562, D7S820, D13S317, D5S818;

D14S548, D7S820, D13S317, D5S818;

D16S490, D7S820, D13S317, D5S818;

D17S1299, D7S820, D13S317, D5S818;

D16S539, D7S820, D13S317, D5S818;

D22S683, D7S820, D13S317, D5S818;

D16S753, D7S820, D13S317, D5S818;

D3S1539, D19S253, D13S317, D20S481;

D3S1539, D19S253, D4S2368, D20S481;

D10S1239, D9S930, D4S2368, D20S481; and

D16S539, D7S820, D13S317, HUMvWFA31.

More preferably, the set of STR loci amplified together is a set of six loci, selected from the sets of loci comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS.

Yet more preferably, the set of STR loci amplified together is a set of seven loci, selected from the sets of loci comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII.

Even more preferably, the set of STR loci amplified together is a set of eight loci, selected from the sets of loci comprising:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII, HUMLIPOL.

The multiplex amplification reaction step of the method is preferably done using a pair of primers flanking each locus in the set of loci co-amplified in the reaction. More preferably, pairs of primers are selected for the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel electrophoresis. Even more preferably, the sequence of one of each pair of primers used in the multiplex amplification reaction is selected from a group of primer sequences consisting of:

SEQ ID NO:1 and SEQ ID NO:2, when one of the loci in the set is D7S820;

SEQ ID NO:3 and SEQ ID NO:4, when one of the loci in the set is D13S317;

SEQ ID NO:5 and SEQ ID NO:6, when one of the loci in the set is D5S818;

SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:49, when one of the loci in the set is D3S1539;

SEQ ID NO:9, SEQ ID NO:10, when one of the loci in the set is D17S1298;

SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO:53, when one of the loci in the set is D20S481;

SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:55, SEQ ID NO:61, when one of the loci in the set is D9S930;

SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54, when one of the loci in the set is D10S1239;

SEQ ID NO:17, SEQ ID NO:18, when one of the loci in the set is D14S118;

SEQ ID NO:19, SEQ ID NO:20, when one of the loci in the set is D14S562;

SEQ ID NO:21, SEQ ID NO:22, when one of the loci in the set is D14S548;

SEQ ID NO:23, SEQ ID NO:24, when one of the loci in the set is D16S490;

SEQ ID NO:25, SEQ ID NO:26, when one of the loci in the set is D16S753;

SEQ ID NO:27, SEQ ID NO:28, when one of the loci in the set is D17S1299;

SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, when one of the loci in the set is D16S539;

SEQ ID NO:31, SEQ ID NO:32, when one of the loci in the set is D22S683;

SEQ ID NO:33, SEQ ID NO:34, when one of the loci in the set is HUMCSF1PO;

SEQ ID NO:35, SEQ ID NO:36, when one of the loci in the set is HUMTPOX;

SEQ ID NO:37, SEQ ID NO:38, when one of the loci in the set is HUMTH01;

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:59, SEQ ID NO:60 when one of the loci in the set is HUMvWFA31;

SEQ ID NO:41, SEQ ID NO:42, when one of the loci in the set is HUMF13A01;

SEQ ID NO:43, SEQ ID NO:44, when one of the loci in the set is HUMFESFPS;

SEQ ID NO:45, SEQ ID NO:46, when one of the loci in the set is HUMBFXIII;

SEQ ID NO:47, SEQ ID NO:48, when one of the loci in the set is HUMLIPOL;

SEQ ID NO:50, SEQ ID NO:51, when one of the loci in the set is D19S253; and

SEQ ID NO:56, SEQ ID NO:57, when one of the loci in the set is D4S2368.

In the method of this invention, the amplified alleles are preferably evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder. The evaluation of alleles is preferably done using polyacrylamide gel electrophoresis to separate the alleles, thereby forming a polyacrylamide gel of separated alleles. The separated alleles in the polyacrylamide gel are preferably determined by visualizing the alleles with an appropriate technique such as silver staining, but more preferably with fluorescent analysis.

Fluorescent analysis is preferably done by labeling one primer of each pair of primers used in the multiplex amplification reaction with a fluorescent label prior to use in the reaction. The fluorescent label used to label each such primer is preferably a fluorescein label or a tetramethyl rhodamine label. Most preferably, at least two different labels are used to label the different primers which are used in the multiplex amplification reaction.

The at least one DNA sample to be analyzed using the method of this invention is preferably isolated from human tissue, preferably tissue selected from the group consisting of blood, semen, vaginal cells, hair, saliva, urine, bone, buccal samples, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

In an alternative embodiment, the invention is a kit for simultaneously analyzing STR sequences in at least three loci, the kit comprising a container which has oligonucleotide primer pairs for co-amplifying a set of at least three short tandem repeat loci, wherein the set of loci are selected from the sets of loci consisting of:

D3S1539, D19S253, D13S317;

D10S1239, D9S930, D20S481;

D10S1239, D4S2368, D20S481;

D10S1239, D9S930, D4S2368;

D16S539, D7S820, D13S317;

D10S1239, D9S930, D13S317;

D3S1539, D7S820, D13S317, D5S818;

D17S1298, D7S820, D13S317, D5S818;
D20S481, D7S820, D13S317, D5S818;
D9S930, D7S820, D13S317, D5S818;
D10S1239, D7S820, D13S317, D5S818;
D14S118, D7S820, D13S317, D5S818;
D14S562, D7S820, D13S317, D5S818;
D14S548, D7S820, D13S317, D5S818;
D16S490, D7S820, D13S317, D5S818;
D17S1299, D7S820, D13S317, D5S818;
D16S539, D7S820, D13S317, D5S818;
D22S683, D7S820, D13S317, D5S818;
D16S753, D7S820, D13S317, D5S818;
D3S1539, D19S253, D13S317, D20S481;
D3S1539, D19S253, D4S2368, D20S481;
D10S1239, D9S930, D4S2368, D20S481;
D16S539, D7S820, D13S317, HUMvWFA31;
D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX;
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS;
D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01;
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII;
D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII, HUMLIPOL.

At least one of the primers in each primer pair included in the kit preferably has a sequence selected from one of the groups of sequences listed under the description of the method of this invention, above.

In yet a third embodiment, the invention is primer sequences and primer pairs for amplifying specific STR loci of human DNA. Use of the primers and primer pairs of this invention for multiplex analysis of human DNA is demonstrated herein below. The primers of this invention are suitable for use in the method of this invention, wherein they can be used in either labeled or unlabeled form depending, as noted above, upon how the amplified alleles are to be determined in the evaluating step of the method.

The present invention, in all its various embodiments described briefly above, provides a high throughput method and materials for the detection and analysis of polymorphic genetic markers using specific combinations of loci and specified conditions. By selection of the appropriate detection technique for the evaluation step, the materials and method of this invention can be used in laboratories which have only a power supply and a standard apparatus for polyacrylamide gel electrophoresis or those which have the latest in equipment for fluorescent gel scanning, e.g., FluorImager™ 575 (Molecular Dynamics, Sunnyvale, Calif.) or the Hitachi FMBIO™ (San Bruno, Calif.) fluorescent scanners or the ABI 373 and ABI Prism™ 377 DNA Sequencers (Applied Biosystems Division, Perkin Elmer, Foster City, Calif.). Thus, the method of the present invention is adaptable for a variety of uses and laboratories.

The approach as specified in the present invention produces a savings in time, labor and materials in the analysis of loci contained within the multiplexes. The method of the present invention allows three or more, even as many as eight or more, loci to be amplified together in one tube using a single amplification reaction, instead of amplifying each locus independently in separate tubes.

The present invention has specific use in the field of forensic analysis, paternity determination, monitoring of bone marrow transplantation, linkage mapping, and detection of genetic diseases and cancers. By allowing three or more loci to be amplified and analyzed simultaneously, the materials and methods of the present invention significantly increase the certainty with which one can match DNA isolated from the blood or other tissues of two different individuals. The need to distinguish accurately between small amounts of tissue of different individuals is particularly acute in forensics applications, where many convictions (and acquittals) turn on DNA typing analysis, including the analysis of STR loci.

Scientists, particularly forensic scientists, have long appreciated the need to analyze multiple polymorphic loci of DNA in order to ensure that a match between two samples of tissue is statistically significant. (Presley, L. A. et al. (1993) "The implementation of the polymerase chain reaction (PCR) HLA DQ alpha typing by the FBI laboratory," in "The Third International Symposium on Human Identification 1992," pp. 245–269; Bever, R. A., et al. (1992) "Characterization of five VNTR loci by Hae III RFLP analysis: application to paternity testing," in "The Second International Symposium on Human Identification 1991," pp. 103–128.) However, until this invention, there were few ways one could simultaneously analyze three or more STR loci in a single reaction. To realize the importance of such multiplexing capabilities, it helps to understand some of the mathematics behind DNA typing analysis.

For purposes of illustration, suppose every STR locus has a genotype (i.e., pattern of two alleles) frequency of one in ten. In other words, suppose that the chance of two randomly selected individuals have a matching type for a single STR is 1/10. However, if two different STR loci are analyzed, the chance of a random match with both systems becomes 1/100. If three STR loci are analyzed, the chances of a random match with each of the three systems become 1/1,000 and so on. Consequently, it is easy to see how increasing the number of STR loci analyzed to any number of loci over three significantly reduces the likelihood of random matches within the general population, thereby increasing the chance one can accurately identify (or eliminate) a suspect in a crime by comparing his type with crime scene evidence. Similar reasoning can be used to conclude that the method of this invention also would increase the likelihood of accurately identifying a suspected father in a paternity case, of correctly matching bone marrow tissue, of developing significant results from linkage mapping studies, and of detecting genetic diseases and cancers.

Further objects, features, and advantages of the invention will be apparent from the following detailed description of the invention and the illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D3S1539, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 1.

FIG. **2** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D17S1298, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 2.

FIG. **3** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of

the loci D17S1298, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 3.

FIG. **4** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D20S481, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 4.

FIG. **5** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D20S481, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 5.

FIG. **6** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D9S930, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 6.

FIG. **7** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D10S1239, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.)in Example 8.

FIG. **8** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D10S1239, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 8.

FIG. **9** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D14S118, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 9.

FIG. **10** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D14S562, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 10.

FIG. **11** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D14S548, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 11.

FIG. **12** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S490, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 12.

FIG. **13** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S753, D7S820, D13S317, and D5S818 as detected with a FluorImager™ T fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 13.

FIG. **14** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D17S1299, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 14.

FIG. **15** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 15.

FIG. **16** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D22S683, D7S820, D13S317, and D5S818 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 16.

FIG. **17** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO") and HUMTPOX ("TPOX") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, Calif.) in Example 17.

FIG. **18** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX") and HUMTH01 ("TH01") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, Calif.) in Example 18.

FIG. **19** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, Calif.) in Example 19.

FIG. **20** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01") and HUMFESFPS ("FESFPS") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, Calif.) in Example 20.

FIG. **21** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01"), HUMFESFPS ("FESFPS") and HUMBFXIII ("F13B") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, Calif.) in Example 21.

FIG. **22** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01"), HUMFESFPS ("FESFPS"), HUMBFXIII ("F13B") and HUMLIPOL ("LPL") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, Calif.) in Example 22.

FIG. **23** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA") as detected with an FMBIO™ Fluorescent Scanner™ (Hitachi Software Engineering, San Bruno, Calif.) in Example 23.

FIG. **24** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D3S1539, D19S253, D13S317 and D20S481 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 24.

FIG. **25** is a laser-printed image showing the fluorescent detection of the products of simultaneous amplification of the loci D10S1239, D9S930, D4S2368 and D20S481 as detected with a FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.) in Example 25.

FIG. **26** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D16S539, D7S820, and D13S317, in Example 26.

FIG. **27** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four

loci, D16S539, D7S820, D13S317 and HUMvWFA31 ("vWA"), in Example 27.

FIG. **28** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D9S930, and D13S317, in Example 28.

FIG. **29** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D9S930, and D4S2368, in Example 29.

FIG. **30** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four loci, D10S1239, D9S930, D4S2368 and D20S481, in Example 30.

FIG. **31** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D3S1539, D19S253 and D13S317, in Example 31.

FIG. **32** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four loci, D3S1539, D19S253, D4S2368 and D20S481, in Example 32.

FIG. **33** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of four loci, D3S1539, D19S253, D13S317 and D20S481, in Example 33.

FIG. **34** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D9S930 and D20S481, in Example 34.

FIG. **35** is a photograph illustrating the silver stain detection of the products of simultaneous amplification of three loci, D10S1239, D4S2368 and D20S481, in Example 35.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

The following definitions are intended to assist in providing a clear and consistent understanding of the scope and detail of the terms:

Allelic ladder: a standard size marker consisting of amplified alleles from the locus.

Allele: a genetic variation associated with a segment of DNA, i.e., one of two or more alternate forms of a DNA sequence occupying the same locus.

Biochemical nomenclature: standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and cytosine (C). Corresponding nucleotides are, for example, deoxyguanosine-5'-triphosphate (dGTP).

DNA polymorphism: the condition in which two or more different nucleotide sequences in a DNA sequence coexist in the same interbreeding population.

Locus (or genetic locus): a specific position on a chromosome. Alleles of a locus are located at identical sites on homologous chromosomes.

Locus-specific primer: a primer that specifically hybridizes with a portion of the stated locus or its complementary strand, at least for one allele of the locus, and does not hybridize efficiently with other DNA sequences under the conditions used in the amplification method.

Polymerase chain reaction (PCR): a technique in which cycles of denaturation, annealing with primer, and extension with DNA polymerase are used to amplify the number of copies of a target DNA sequence by approximately 10^6 times or more. The polymerase chain reaction process for amplifying nucleic acid is covered by U. S. Pat. Nos. 4,683,195 and 4,683,202, which are incorporated herein by reference for a description of the process.

Polymorphic short tandem repeat loci: STR loci in which the number of repetitive sequence elements (and net length of sequence) in a particular region of genomic DNA varies from allele to allele, and from individual to individual.

Polymorphism information content (PIC): a measure of the amount of polymorphism present at a locus (Botstein et al., 1980). PIC values range from 0 to 1.0, with higher values indicating greater degrees of polymorphism. This measure generally displays smaller values than the other commonly used measure, i.e., heterozygosity. For markers that are highly informative (heterozygosities exceeding about 70%), the difference between heterozygosity and PIC is slight.

Primary reaction: initial reaction using the purified human genomic DNA as template for the PCR.

Primers: two single-stranded oligonucleotides or DNA fragments which hybridize with opposing strands of a locus such that the 3' termini of the primers are in closest proximity.

Primer pair: two primers including, primer 1 that hybridizes to a single strand at one end of the DNA sequence to be amplified and primer 2 that hybridizes with the other end on the complementary strand of the DNA sequence to be amplified.

Primer site: the area of the target DNA to which a primer hybridizes.

Secondary reaction: reamplification with the same or different primer pair using a dilution of the primary reaction as template for the PCR.

Short tandem repeat loci (STR loci): regions of the human genome which contain short, repetitive sequence elements of 3 to 7 base pairs in length.

B. Selection of Multiplex Reaction Components

The method of the present invention contemplates selecting an appropriate set of loci, primers, and amplification protocols to generate amplified alleles from multiple co-amplified loci which either do not overlap in size or which are labeled in some way to make the amplified alleles which do overlap in size distinguishable from one another. In addition, this method contemplates the selection of short tandem repeat loci which are compatible for use with a single amplification protocol. The specific combinations of loci described herein are unique in this application. Combinations of loci may be rejected for either of the above two reasons, or because, in combination, one or more of the loci do not produce adequate product yield, or fragments which do not represent authentic alleles are produced in this reaction.

Successful combinations in addition to those disclosed herein can be generated by trial and error of locus combinations, by selection of primer pair sequences, and by adjustment of primer concentrations to identify an equilibrium in which all included loci may be amplified. Once the method and materials of this invention are disclosed, various methods of selecting loci, primer pairs, and amplification techniques for use in the method and kit of this invention are likely to be suggested to one skilled in the art. All such methods are intended to be within the scope of the appended claims.

Of particular importance in the practice of the method of this invention is the size range of amplified alleles produced from the individual loci which are amplified together in the multiplex amplification reaction step. For ease of analysis with current technologies, systems which can be detected by amplification of fragments smaller than 500 bases are most preferable. The most preferable combinations of loci, primers, and amplification techniques are described in the Summary of the Invention section, above.

Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplifica-

tion at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex. Synthesis of the primers used in the present method can be conducted using any standard procedure for oligonucleotide synthesis known to those skilled in the art.

C. Use of Multiplexes of Three Loci to Develop Multiplexes Using More than Three Loci

Any one of a number of different techniques can be used to select the set of STR loci to be analyzed using a method of the present invention. One preferred technique for developing useful sets of loci for use in this method of analysis is described below. Once a multiplex containing three loci is developed, it may be used as a core to create multiplexes containing more than three loci. New combinations are created including the first three loci. For example, the core multiplex containing loci D7S820, D13S317, and D5S818 was used to generate derivative multiplexes of D16S539, D7S820, D13S317, and D5S818; HUMCSF1PO, HUMTPOX, D16S539, D7S820, D13S317, and D5S818; HUMCSF1PO, HUMTPOX, HUMTH01, D16S539, D7S820, D13S317, and D5S818; and HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31, D16S539, D7S820, D13S317, and D5S818.

It is contemplated that core sets of loci can be used to generate other appropriate derivative sets of STR loci for multiplex analysis using the method of this invention. Regardless of what method is used to select the loci analyzed using the method of the present invention, all the loci selected for multiplex analysis should share the following characteristics: (1) they should produce minimal slippage (e.g., from misreading the repeat sequence during an amplification step), (2) few if any artifacts due to the addition or deletion of a base to the amplified alleles during the multiplex amplification step, (3) few if any artifacts due to premature termination of amplification reactions by a polymerase, and (4) no "trailing" bands of smaller molecular weight from consecutive single base deletions below a given authentic amplified allele. See, e.g., Schumm et al., "Development of Nonisotopic Multiplex Amplification Sets for Analysis of Polymorphic STR Loci," *Fourth International Symposium on Human Identification* 1993, pp. 177–187 (pub. by Promega Corp., 1993).

D. Preparation of DNA Samples

Samples of human genomic DNA can be prepared for use in the method of this invention using any method of DNA preparation which is compatible with the amplification of a single locus. Many such methods are suitable for use in preparing genomic DNA samples for use in the method of this invention, including, but not limited to, the methods of DNA sample preparation described by Patel, P. I., et al. (1984) "Organization of the HPRT gene and related sequences in the human genome," *Somat Cell Mol Genet* 10: 483–493, and Gill, P., et al. (1985) "Forensic application of DNA 'fingerprints'," *Nature* 318: 577–579.

DNA concentrations can be measured prior to use in the method of the present invention, using any standard method of DNA detection. However, the DNA concentration is preferably measured fluorometrically using a measurement technique such as that described by Brunk C. F., et al. 4 (1979) "Assay for nanogram quantities of DNA in cellular homogenates," *Anal Biochem* 92: 497–500. The DNA concentration is more preferably measured by comparison of the amount of hybridization of DNA standards with a human-specific probe such as that described by Waye et al. (1979) Waye, J. S., et al. (1991) "Sensitive and specific quantification of human genomic deoxyribonucleic acid (DNA) in forensic science specimens: casework examples," *J. Forensic Sci.,* 36:1198–1203. Use of too much template DNA in the amplification reactions can produce artifacts which appear as extra bands which do not represent true alleles.

E. Amplification of DNA

Once a sample of human genomic DNA is isolated, and its concentration determined as described above, the targeted loci can be co-amplified in the multiplex amplification step of the present method. Any one of a number of different amplification methods can be used to amplify the loci, including, but not limited to, polymerase chain reaction (PCR) (Saiki, R. K., et al. (1985) "Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia," *Science* 230: 1350–1354), transcription based amplification (Kwoh, D. Y., and Kwoh, T. J. (1990) "Target amplification systems in nucleic acid-based diagnostic approaches," *American Biotechnology Laboratory, October,* 1990) and strand displacement amplification (SDA) (Walker, G. T., et al. (1992) "Isothermal in vitro amplification of DNA by a restriction enzyme-DNA Polymerase system," *Proc. Natl. Acad. Sci., U.S.A.* 89: 392–396). Preferably, the DNA sample is subjected to PCR amplification using primer pairs and thermocycling conditions specific to each locus in the set. Reference is made to the Sequence Listing at the end of this specification for details of the primer sequences used in the Examples below, some of which sequences are alternative embodiments of this invention.

Details of the most preferred amplification protocol for each of the most preferred combinations of loci for use in the method of this invention are given in the examples below. Reference is also made to the examples for additional details of the specific procedure relating to each multiplex. The sequences of the locus-specific primers used in the examples include a number of nucleotides which, under the conditions used in the hybridization, are sufficient to hybridize with an allele of the locus to be amplified and to be essentially free from amplification of alleles of other loci. Reference is made to U.S. Pat. No. 5,192,659 to Simons, the teaching of which is incorporated herein by reference for a more detailed description of locus-specific primers.

F. Separation and Detection of DNA Fragments

Once a set of amplified alleles is produced from the multiplex amplification step of the present method, the amplified alleles are evaluated. The evaluation step of this method can be accomplished by any one of a number of different means, the most preferred of which are described below.

Electrophoresis is preferably used to separate the products of the multiplex amplification reaction, more preferably denaturing polyacrylamide gel electrophoresis (see, e.g., Sambrook, J. et al. (1989) *In Molecular Cloning-A Laboratory Manual,* 2nd edition, Cold Spring Harbor Laboratory Press, pp. 13.45–13.57). The most preferred gel preparation and electrophoresis procedures and conditions for use in the evaluating step of the method of this invention are described in Example 1. Separation of DNA fragments in a denaturing polyacrylamide gel occurs based on fragment size.

Once the amplified alleles are separated in a polyacrylamide gel, the alleles and any other DNA in the gel (e.g., DNA markers or an allelic ladder) can then be visualized and analyzed. Visualization of the DNA in the gel can be accomplished using any one of a number of prior art techniques, including silver staining or reporters such as radioisotopes, fluorescers, chemiluminescers and enzymes

in combination with detectable substrates. Silver staining is a preferred method of visualizing the alleles in the gel (see, e.g., Bassam, B. J., et al. (1991) "Fast and sensitive silver staining of DNA in polyacrylamide gels," *Anal. Biochem.* 196: 80–83). A more preferred method is the use of radioactively-labeled (see, e.g., Hammond et al., (1994)) or fluorescently-labeled (see, e.g., Schumm et al., (1994)) primers for each locus in the multiplexing reaction followed by detection of the labeled products using an autoradiogram or fluorometric detector, respectively. All three references, cited above, which describe prior art methods of visualizing alleles, are incorporated by reference herein.

The alleles present in the DNA sample are preferably determined by comparison to a size standard such as a DNA marker or a locus-specific allelic ladder to determine the alleles present at each locus within the sample. The most preferred size marker for evaluation of a multiplex amplification containing two or more polymorphic STR loci consists of a combination of allelic ladders for each of the loci being evaluated. See, e.g., description of allelic ladders and method of ladder construction in Schumm et al., supra, at p. 178.

The preferred size marker for evaluation of a multiplex amplification containing two or more polymorphic STR loci which are generated using fluorescently-labeled primers for each locus consists of a combination of fluorescently-labeled allelic ladders for the loci being evaluated. Id.

Following the construction of allelic ladders for individual loci, they may be mixed and loaded for gel electrophoresis at the same time as the loading of amplified samples occurs. Each allelic ladder co-migrates with alleles in the sample from the corresponding locus.

A permanent record of the data can be generated using Automatic Processor Compatible (APC) film (STR systems manual #TMD004, available from Promega Corporation, Madison, Wis.) or with use of a fluorescent detection instrument (STR systems manual #TMD006, also available from Promega Corporation, Madison, Wis.).

G. Preferred Detection Technique: Fluorescent Detection

In one of the most preferred embodiments of the method of this invention, fluorescent detection is used to evaluate the amplified alleles in the mixture produced by the multiplex amplification reaction. Below is a brief summary of how that method of detection preferably is practiced.

With the advent of automated fluorescent imaging, faster detection and analysis of multiplex amplification products can be achieved. For fluorescent analyses, one fluorescein-ated primer can be included in the amplification of each locus. Descriptions of the use of two preferred species of fluorescent labeled primers, fluorescein-labeled (FL-) and tetramethyl rhodamine-labeled (TMR-) primers are included in the examples, below. Separation of the amplified fragments produced using such labeled primers is achieved in precisely the same manner as with the silver stain detection method. The resulting gel can be analyzed using a FluorImager™ analyzer (commercially available from Molecular Dynamics, Sunnyvale, Calif.) or FMBIO™ (commercially available from Hitachi Corporation, San Bruno, Calif.), which scans the gel and digitizes the data in a very short time, e.g., three to twenty minutes.

In summary, the method of this invention is most preferably practiced using fluorescent detection at the evaluating step. In this preferred method of detection, one of each pair of primers used in the multiplex amplification reaction has a fluorescent label attached thereto, and as a result, the amplified alleles produced from the amplification reaction are fluorescently labeled. In this most preferred embodiment of the invention, the amplified alleles are subsequently separated on a polyacrylamide gel and the separated alleles visualized and analyzed using a fluorescent image analyzer.

Fluorescent detection is preferred over radioactive methods of labeling and detection, because it does not require the use of radioactive materials, and all the regulatory and safety problems which accompany the use of such materials.

Fluorescent detection is also preferred over other non-radioactive methods of detection, such as silver staining, because fluorescent methods of detection generally reveal fewer gel artifacts than staining. The smaller number of gel artifacts are probably due, to a large extent, to the fact that only amplified fragments of DNA with labels attached are detected in fluorescent detection, while every amplified fragment of DNA produced from the multiplex amplification reaction is stained and detected using the silver staining method of detection. Polyacrylamide gels stained with silver stain also have a considerably higher general background due to nonspecific binding of silver stain to the gel itself, reducing the sensitivity with which individual bands of DNA can be detected within the gel. Silver staining and fluorescent methods of detection are compared in two sets of examples, hereinbelow.

H. Kit

The present invention is also directed to kits that utilize the process described above. A basic kit comprises a container having one or more locus-specific primers for each locus. Instructions for use optionally may be included.

Other optional kit components may include an allelic ladder directed to each of the specified loci, a sufficient quantity of enzyme for amplification, amplification buffer to facilitate the amplification, loading solution for preparation of the amplified material for gel electrophoresis, human genomic DNA as a template control, a size marker to insure that materials migrate as anticipated in the gel, and a protocol and manual to educate the user and to limit error in use. The amounts of the various reagents in the kits also can be varied depending upon a number of factors, such as the optimum sensitivity of the process. It is within the scope of this invention to provide test kits for use in manual applications or test kits for use with automated detectors or analyzers.

EXAMPLES

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the disclosure or protection granted by the patent.

Genomic DNA isolation and quantitation were performed essentially as described by Puers, C., et al. (1993) "Identification of repeat sequence heterogeneity at the polymorphic short tandem repeat locus HUMTH01 $[AATG]_n$ and reassignment of alleles in population analysis by using a locus-specific allelic ladder," *Am. J. Hum. Genet.* 53: 953–958. These methods are generally known to those skilled in the art and are preferred, but not required, for application of the invention.

Amplification products were separated by electrophoresis through a 0.4 mm thick 4% denaturing polyacrylamide gel (19:1 ratio of acrylamide to bis-acrylamide) which contained 7M urea (Sambrook et al., (1989)), and which was chemically cross-linked to a glass plate (Kobayashi, Y. (1988) "A method to cast thin sequencing gels," *BRL Focus* 10: 73–74) in cases involving silver stain analysis. No such cross-linking was employed in cases involving fluorescent analysis. DNA samples were mixed with 2.5 μl of a loading

solution (10 MM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured at 95° C. for 2 min., and chilled on ice prior to loading.

Once separated by polyacrylamide gel electrophoresis, the amplified reaction products and DNA size marker controls were detected using silver staining, fluorescent detection, radioactive detection, or a combination of the above detection methods. In some Examples, the reaction products and size markers in the gel were detected by silver staining using a standard method of staining and detection described in the prior art. (See, e.g., Bassam et al., (1991).) Permanent images of the stained gels were obtained by exposure to Automatic Processor Compatible Film (APC Film, Promega Corporation, Cat. No. DQ4411). In other Examples, detection was performed by fluorescent scanning, using a method described in the prior art (Schumm et al., (1994)).

Each example below is an example of the use of the method of this invention, and in some cases, an example of the use of one or more of the primers of this invention to determine simultaneously the alleles present in at least three short tandem repeat loci from one or more DNA samples. Tables 1 and 2 summarize which set of loci was co-amplified in the multiplex amplification reaction described in each Example below. The two tables also indicate which primer pair was used to amplify each such locus in each such multiplex reaction. Table 1 lists all the Examples where fluorescent scanning was used to detect the amplified alleles from the multiplex reactions described therein, while Table 2 lists the Examples where silver staining was used to detect the amplified alleles.

One primer of each primer pair listed on Table 1 was fluorescently labeled prior to being used in the multiplex amplification reaction. In some cases, a different label was used to label primers to different loci, such that the alleles produced using the different primers could be distinguished from one another when scanned by the fluorescent scanner used in the Examples below. Two different fluorescent labels were used in the Examples below, described as "FL" to indicate fluorescein-labeled and "TMR" to indicate tetramethyl rhodamine-labeled in Table 1, below. Table 1 also indicates which primer of each pair of primers used in the multiplex amplification reaction was so labeled in each example (e.g., "FL-2" means the primer with SEQ ID NO:2 was labeled at its 5' end with fluorescein prior to being used in the multiplex amplification reaction).

The same FL and TMR abbreviations are used in the Examples below. However, there the label abbreviation is placed immediately before the SEQ ID NO of the labeled primer used in the amplification reaction described therein (e.g., "FL-SEQ ID NO:2" instead of "FL-2").

In four pairs of Examples below (Examples 2 and 3, 4 and 5, 7 and 8, and 19 and 23), the same set of loci were analyzed using the same set of primers and the same fluorescent labels covalently attached to one of each pair of primers for each STR locus analyzed. However, a different set of primers was labeled in each of the Examples. These pairs of Examples are included herein to demonstrate that the same low background and identical allelic determination results can be obtained from the same set of primers using fluorescent labeling as a method of detection, no matter which of the primers of a primer pair is labeled prior to being used in a multiplex amplification reaction of the method of this invention.

TABLE 1

Example	Loci Amplified	Primer Pair: SEQ ID NO:'s	Fluorescent Label(s) Used
1	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D3S1539	7, 8	FL-8
2	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D17S1298	9, 10	FL-10
3	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D17S1298	9, 10	FL-9
4	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D20S481	11, 12	FL-12
5	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D20S481	11, 12	FL-11
6	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D9S930	13, 14	FL-14
7	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D10S1239	15, 16	FL-16
8	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D10S1239	15, 16	FL-15
9	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D14S118	17, 18	FL-18
10	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D14S562	19, 20	FL-19
11	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D14S548	21, 22	FL-22
12	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S490	23, 24	FL-23
13	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S753	25, 26	FL-26
14	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D17S1299	27, 28	FL-28
15	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S539	29, 30	FL-30
16	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D22S683	31, 32	FL-32
17	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S539	29, 30	FL-30
	HUMCSF1PO	33, 34	TMR-33
	HUMTPOX	35, 36	TMR-36
18	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S539	29, 30	FL-30
	HUMCSF1PO	33, 34	TMR-33
	HUMTPOX	35, 36	TMR-36
	HUMTH01	37, 38	TMR-38

TABLE 1-continued

Example	Loci Amplified	Primer Pair: SEQ ID NO:'s	Fluorescent Label(s) Used
19	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S539	29, 30	FL-30
	HUMCSF1PO	33, 34	TMR-33
	HUMTPOX	35, 36	TMR-36
	HUMTH01	37, 38	TMR-38
	HUMvWFA31	39, 40	TMR-40
20	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S539	29, 30	FL-30
	HUMF13A01	41, 42	TMR-41
	HUMFESFPS	43, 44	TMR-43
21	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S539	29, 30	FL-30
	HUMF13A01	41, 42	TMR-41
	HUMFESFPS	43, 44	TMR-43
	HUMBFXIII	45, 46	TMR-45
22	D7S820	1, 2	FL-2
	D13S317	3, 4	FL-4
	D5S818	5, 6	FL-6
	D16S539	29, 30	FL-30
	HUMF13A01	41, 42	TMR-41
	HUMFESFPS	43, 44	TMR-43
	HUMBFXIII	45, 46	TMR-45
	LIPOL	47, 48	TMR-47
23	D7S820	1, 2	TMR-2
	D13S317	3, 4	TMR-4
	D5S818	5, 6	TMR-6
	D16S539	29, 30	TMR-30
24	D3S1539	7, 49	FL-49
	D19S253	50, 51	FL-50
	D13S317	3, 4	FL-4
	D20S481	52, 53	FL-53
25	D10S1239	15, 54	FL-15
	D9S930	55, 14	FL-14
	D4S2368	56, 57	FL-57
	D20S481	52, 53	FL-53

Note that in a few cases, the same set of loci and same set of primer pairs appear in Table 1 and in Table 2. In such cases, the same set of alleles were analyzed using fluorescent detection and silver staining, respectively. Two such cases of duplicate sets are provided herein, Examples 24 and 33, and Examples 25 and 30. These examples clearly illustrate that the same results can be obtained with either method.

TABLE 2

Example	Loci Amplified	Primer Pair: SEQ ID NO:'s
26	D16S539	29, 58
	D7S820	1, 2
	D13S317	3, 4
27	D16S539	29, 30
	D7S820	1, 2
	D13S317	3, 4
	HUMvWFA31	59, 60
28	D10S1239	15, 54
	D9S930	55, 61
	D13S317	31, 4
29	D10S1239	15, 54
	D9S930	55, 61
	D4S2368	56, 57
30	D10S1239	15, 54
	D9S930	55, 14
	D4S2368	56, 57
	D20S481	52, 53

TABLE 2-continued

Example	Loci Amplified	Primer Pair: SEQ ID NO:'s
31	D3S1539	7, 49
	D19S253	50, 51
	D13S317	3, 4
32	D3S1539	7, 49
	D19S253	50, 51
	D4S2368	56, 57
	D20S481	52, 53
33	D3S1539	7, 49
	D19S253	50, 51
	D13S317	3, 4
	D20S481	52, 53
34	D10S1239	15, 54
	D9S930	55, 14
	D20S481	52, 53
35	D10S1239	15, 54
	D4S2368	56, 57
	D20S481	52, 53

Example 1

Flourescent Detection of Multiplex Amplification of Loci D3S1539, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D3S1539, D7S820, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Tag DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.25 μM each D3S1539 primers 1 [SEQ ID NO:7] and 2 [FL-SEQ ID NO:8], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.219 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **1** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D3S1539, D7S820, D13S317, and D5S818.

Example 2

Fluorescent Detection of Multiplex Amplification of Loci D17S1298. D7S820. D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D17S1298, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA

Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.25 μM each D17S1298 primers 1 [SEQ ID NO:9] and 2 [FL-SEQ ID NO:10], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.219 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **2** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D17S1298, D7S820, D13S317, and D5S818.

Example 3

Fluorescent Detection of Multiplex Amplification of Loci D17S1298, D7S820, D13S317, and D5S818

The loci D17S1298, D7S820, D13S317, and D5S818 were amplified as described in Example 2 except that SEQ ID NO:9 was replaced with FL-SEQ ID NO:9 and FL-SEQ ID NO:10 was replaced with SEQ ID NO:10.

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **3** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D17S1298, D7S820, D13S317, and D5S818.

Example 4

Fluorescent Detection of Multiplex Amplification of Loci D20S481, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D20S481, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.25 μM each D20S481 primers 1 [SEQ ID NO:11] and 2 [FL-SEQ ID NO:12], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.219 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **4** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D20S481, D7S820, D13S317, and D5S818.

Example 5

Fluorescent Detection of Multiplex Amplification of Loci D20S481, D7S820. D13S317, and D5S818

The loci D20S481, D7S820, D13S317, and D5S818 were amplified as described in Example 4 except that SEQ ID NO:11 was replaced with FL-SEQ ID NO:11 and FL-SEQ ID NO:12 was replaced with SEQ ID NO:12.

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **5** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D20S481, D7S820, D13S317, and D5S818.

Example 6

Fluorescent Detection of Multiplex Amplification of Loci D9S930. D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D9S930, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.70 μM each D9S930 primers 1 [SEQ ID NO:13] and 2 [FL-SEQ ID NO:14], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3) and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **6** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D9S930, D7S820, D13S317, and D5S818.

Example 7

Fluorescent Detection of Multiplex Amplification of Loci D10S1239, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D10S1239, D7S820,

D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.75 μM each D10S1239 primers 1 [SEQ ID NO:15] and 2 [FL-SEQ ID NO:16], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **7** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D10S1239, D7S820, D13S317, and D55818.

Example 8

Fluorescent Detection of Multiplex Amplification of Loci D10S1239. D7S820, D13S317, and D5S818

The loci D10S1239, D7S820, D13S317, and D5S818 were amplified as described in Example 7 except that SEQ ID NO:15 was replaced with FL-SEQ ID NO:15 and FL-SEQ ID NO:16 was replaced with SEQ ID NO:16.

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **8** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D10S1239, D7S820, D13S317, and D5S818.

Example 9

Fluorescent Detection of Multiplex Amplification of Loci D14S118, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D14S118, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.50 μM each D14S118 primers 1 [SEQ ID NO:17] and 2 [FL-SEQ ID NO:18], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **9** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D14S118, D7S820, D13S317, and D5S818.

Example 10

Fluorescent Detection of Multiplex Amplification of Loci D14S562. D7S820 D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D14S562, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.50 μM each D14S562 primers 1 [FL-SEQ ID NO:19] and 2 [SEQ ID NO:20], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **10** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D14S562, D7S820, D13S317, and D5S818.

Example 11

Fluorescent Detection of Multiplex Amplification of Loci D14S548, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D14S548, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.50 μM each D14S548 primers 1 [SEQ ID NO:21] and 2 [FL-SEQ ID NO:22], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **11** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D14S548, D7S820, D13S317, and D5S818.

Example 12

Fluorescent Detection of Multiplex Amplification of Loci D16S490, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D16S490, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.50 μM each D16S490 primers 1 [FL-SEQ ID NO:23] and 2 [SEQ ID NO:24], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **12** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S490, D7S820, D13S317, and D5S818.

Example 13

Fluorescent Detection of Multiplex Amplification of Loci D16S753, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D16S753, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.50 μM each D16S753 primers 1 [SEQ ID NO:25] and 2 [FL-SEQ ID NO:26], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **13** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S753, D7S820, D13S317, and D5S818.

Example 14

Fluorescent Detection of Multiplex Amplification of Loci D17S1299. D7S820. D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D17S1299, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.50 μM each D17S1299 primers 1 [SEQ ID NO:27] and 2 [FL-SEQ ID NO:28], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **14** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D17S1299, D7S820, D13S317, and D5S818.

Example 15

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster

City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.60 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.50 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **15** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, and D5S818.

Example 16

Fluorescent Detection of Multiplex Amplification of Loci D22S683, D7S820, D13S317, and D5S818

In this example, a DNA template was amplified simultaneously at the individual loci D22S683, D7S820, D13S317, and D5S818 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.50 μM each D22S683 primers 1 [SEQ ID NO:31] and 2 [FL-SEQ ID NO:32], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.375 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 55 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **16** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D22S683, D7S820, D13S317, and D5S818.

Example 17

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO and HUMTPOX

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO and HUMTPOX in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.06 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Twelve amplification primers were used in combination, including 0.65 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], 0.325 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.22 μM each D13S317 primers 1 [SEQ. ID NO:3] and 2 [FL-SEQ ID NO:4], 0.55 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], 0.40 μM each HUMCSF1PO primers 1 [TMR-SEQ ID NO:33] and 2 [SEQ ID NO:34], 0.40 μM each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [TMR-SEQ ID NO:36].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, Calif.).

Reference is made to FIG. **17** which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes **1** to **4** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO") and HUMTPOX ("TPOX").

Example 18

Fluorescent Detection of Multiplex Amplification of Loci D16S539. D7S820, D13S317, D5S818. HUMCSF1PO, HUMTPOX and HUMTH01

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX and HUMTH01 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0. 07 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Fourteen amplification primers were used in combination, including 0.75 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], 0.40 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.30 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.60 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], 0.30 μM each HUMCSF1PO primers 1 [TMR-SEQ ID NO:33] and 2 [SEQ ID NO:34], 0.40 μM each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [TMR-SEQ ID NO:35], 0.40 μM each HUMTH01 primers 1 [SEQ ID NO:37] and 2 [TMR-SEQ ID NO:38].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at

40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, Calif.).

Reference is made to FIG. **18** which displays the amplified fragments of each locusin separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX") and HUMTH01 ("TH01").

Example 19

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.08 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Sixteen amplification primers were used in combination, including 0.75 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], 0.40 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.30 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.60 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], 0.30 μM each HUMCSF1PO primers 1 [TMR-SEQ ID NO:33] and 2 [SEQ ID NO:34], 0.40 μM each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [TMR-SEQ ID NO:36], 0.40 μM each HUMTH01 primers 1 [SEQ ID NO:37] and 2 [TMR-SEQ ID NO:38], 0.40 μM each HUMvWFA31 primers 1 [SEQ ID NO:39] and 2 [TMR-SEQ ID NO:40].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, Calif.).

Reference is made to FIG. **19** which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA").

Example 20

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 and HUMFESFPS

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 and HUMFESFPS in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.06 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Twelve amplification primers were used in combination, including 0.75 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], 0.40 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.30 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.60 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], 0.10 μM each HUMF13A01 primers 1 [TMR-SEQ ID NO:41] and 2 [SEQ ID NO:42], 1.0 μM each HUMFESFPS primers 1 [TMR-SEQ ID NO:43] and 2 [SEQ ID NO:44].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, Calif.).

Reference is made to FIG. **20** which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMF13A10 ("F13A01") and HUMFESFPS ("FESFPS").

Example 21

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMF13A01. HUMFESFPS and HUMBFXIII

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS and HUMBFXIII in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.07 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Fourteen amplification primers were used in combination, including 0.75 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], 0.40 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.30 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.60 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], 0.10 μM each HUMF13A01 primers 1 [TMR-SEQ ID NO:41] and 2 [SEQ ID NO:42], 1.0 μM each HUMFESFPS primers 1 [TMR-SEQ ID NO:43] and 2 [SEQ ID NO:44], 0.50 μM each HUMBFXIII primers 1 [TMR-SEQ ID NO:45] and 2 [SEQ ID NO:46].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FNBIOFluorImager™ (Hitachi Software Engineering, San Bruno, Calif.).

Reference is made to FIG. **21** which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("F13A01"), HUMFESFPS ("FESFPS") and HUMBFXIII ("F13B").

Example 22

Fluorescent Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317, D5S818, HUMF13A10, HUMFESFPS, HUMBFXIII and HUMLIPOL

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMF13A10, HUMFESFPS, HUMBFXIII and HUMLIPOL in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.08 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Sixteen amplification primers were used in combination, including 0.75 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:30], 0.40 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [FL-SEQ ID NO:2], 0.30 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.60 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [FL-SEQ ID NO:6], 0.10 μM each HUMF13A01 primers 1 [TMR-SEQ ID NO:41] and 2 [SEQ ID NO:42], 1.0 μM each HUMFESFPS primers 1 [TMR-SEQ ID NO:43] and 2 [SEQ ID NO:44], 0.50 μM each HUMBFXIII primers 1 [TMR-SEQ ID NO:45] and 2 [SEQ ID NO:46], 0.20 μM each HUMLIPOL primers 1 [TMR-SEQ ID NO:47] and 2 [SEQ ID NO:48].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, Calif.).

Reference is made to FIG. **22** which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMF13A01 ("HUMF13A01"), HUMFESFPS ("FESFPS"), HUMBFXIII ("F13B") and HUMLIPOL ("LPL").

Example 23

Fluorescent Detection of Multiplex Amplification of Loci D16S539. D7S820. D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31

In this example, a DNA template was amplified simultaneously at the individual loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.08 U Tag DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Sixteen amplification primers were used in combination, including 0.75 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [TMR-SEQ ID NO:30], 0.40 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [TMR-SEQ ID NO:2], 0.30 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [TMR-SEQ ID NO:4], 0.60 μM each D5S818 primers 1 [SEQ ID NO:5] and 2 [TMR-SEQ ID NO:6], 0.40 μM each HUMCSF1PO primers 1 [FL-SEQ ID NO:33] and 2 [SEQ ID NO:34], 0.50 μM each HUMTPOX primers 1 [SEQ ID NO:35] and 2 [FL-SEQ ID NO:36], 0.20 μM each HUMTH01 primers 1 [SEQ ID NO:37] and 2 [FL-SEQ ID NO:38], 0.55 μM each HUMvWFA31 primers 1 [SEQ ID NO:39] and 2 [FL-SEQ ID NO:40].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FMBIOFluorImager™ (Hitachi Software Engineering, San Bruno, Calif.).

Reference is made to FIG. **23** which displays the amplified fragments of each locus in separate 505 nm and 625 nm scans of the same gel revealing fluorescein-labeled and tetramethyl-rhodamine labeled material, respectively. Lanes **1** to **3** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317, D5S818, HUMCSF1PO ("CSF1PO"), HUMTPOX ("TPOX"), HUMTH01 ("TH01") and HUMvWFA31 ("vWA").

Example 24

Fluorescent Detection of Multiplex Amplification of Loci D3S1539, D19S253, D13S317, and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci D3S1539, D19S253, D13S317 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.75 μM each D3S1539 primers 1 [SEQ ID NO:7] and 2 [FL-SEQ ID NO:49], 0.75 μM each D19S253 primers 1 [FL-SEQ ID NO:50] and 2 [SEQ ID NO:51], 0.50 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO:4], 0.50 μM each D20S481 primers 1 [SEQ ID NO:52] and 2 [FL-SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the

fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **24** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253, D13S317 and D20S481.

Example 25

Fluorescent Detection of Multiplex Amplification of Loci D10S1239, D9S930, D4S2368, and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci D10S1239, D9S930, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.30 μM each D10S1239 primers 1 [FL-SEQ ID NO:15] and 2 [SEQ ID NO:54], 0.40 μM each D9S930 primers 1 [SEQ ID NO:55] and 2 [FL-SEQ ID NO:14], 0.50 μM each D4S2368 primers 1 [SEQ ID NO:56] and 2 [FL-SEQ ID NO:57], 0.50 μM each D20S481 primers 1 [SEQ ID NO:52] and 2 [FL-SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by detection of the fluorescent signals using the FluorImager™ fluorescent scanner (Molecular Dynamics, Sunnyvale, Calif.).

Reference is made to FIG. **25** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930, D4S2368 and D20S481.

Example 26

Silver Detection of Multiplex Amplification of Loci D16S539, D7S820 and D13S317

In this example, a DNA template was amplified simultaneously at the individual loci, D16S539, D7S820, and D13S317 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 to 25 ng template, and 0.03 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min, followed by 1 cycle of 60° C. for 30 min.

Six amplification primers were used in combination, including 0.5 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [SEQ ID NO:58], 0.5 μM each D7S820 primers 1 [SEQ ID NO:1] and 2 [SEQ ID NO:2], 0.5 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **26** which displays the amplified fragments of each locus. Lanes **1** to **4** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, and D13S317 and lane **5** displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

Example 27

Silver Detection of Multiplex Amplification of Loci D16S539, D7S820, D13S317 and HUMvWFA31

In this example, a DNA template was amplified simultaneously at the individual loci, D16S539, D7S820, D13S317 and HUMvWFA31 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1. 5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min, followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.3 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [SEQ ID NO:30], 0.3 μM each D7S820 primers 1 [SEQ ID NO:13 and 2 [SEQ ID NO:2], 0.5 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 (SEQ ID NO:4], 0.5 μM each HUMvWFA31 primers 1 [SEQ ID NO:59] and 2 [SEQ ID NO:60].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 32 cm gel for 50 min. at 40 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **27** which displays the amplified fragments of each locus. Lanes **1** to **3** contain DNA samples simultaneously co-amplified for the loci D16S539, D7S820, D13S317 and HUMvWFA31 ("vWA").

Example 28

Silver Detection of Multiplex Amplification of Loci D10S1239, D9S930, and D13S317

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D9S930, and D13S317 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using undetermined ng template, and 0.03 U Tag DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 minutes.

Six amplification primers were used in combination, including 1.0 μM each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 0.3 μM each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:61], 0.5 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 60 min. at

60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **28** which displays the amplified fragments of each locus. Lanes **1** to **3** contain DNA samples simultaneously co-amplified for the loci D1OS1239, D9S930, and D13S317.

Example 29

Silver Detection of Multiplex Amplification of Loci D10S1239, D9S930, and D4S2368

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D9S930, and D4S2368 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using undetermined ng template, and 0.03 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Six amplification primers were used in combination, including 1.0 μM each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 0.3 μM each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:61], 0.15 μM each D4S2368 primers 1 (SEQ ID NO:56] and 2 [SEQ ID NO:57].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 60 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **29** which displays the amplified fragments of each locus. Lanes **1** to **6** contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930, and D4S2368.

Example 30

Silver Detection of Multiplex Amplification of Loci D10S1239, D9S930, D4S2368 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D9S930, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.55 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using undetermined ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 1.0 μM each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 4.0 μM each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:14], 0.2 μM each D4S2368 primers 1 [SEQ ID NO:56] and 2 [SEQ ID NO:57] and 0.2 μM each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 67 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **30** which displays the amplified fragments of each locus. Lanes **1** to **4** contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930, D4S2368 and D20S481.

Example 31

Silver Detection of Multiplex Amplification of Loci D3S1539, D19S253 and D13S317

In this example, a DNA template was amplified simultaneously at the individual loci, D3S1539, D19S253 and D13S317 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5.0 ng template, and 0.03 U Tag DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min.

Six amplification primers were used in combination, including 1.0 μM each D3S1539 primers 1 [SEQ ID NO:7] and 2 [SEQ ID NO:49], 1.0 μM each D19S253 primers 1 [SEQ ID NO:50] and 2 [SEQ ID NO:51], 0.5 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [SEQ ID NO:4].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 65 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **31** which displays the amplified fragments of each locus. Lanes **1** to **4** contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253 and D13S317 and lane **5** displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

Example 32

Silver Detection of Multiplex Amplification of Loci D3S1539, D19S253, D4S2368 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D3S1539, D19S253, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 5 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min.

Eight amplification primers were used in combination, including 1.0 μM each D3S1539 primers 1 [SEQ ID NO:7] and 2 [SEQ ID NO:49], 0.5 μM each D19S253 primers 1 [SEQ ID NO:50] and 2 [SEQ ID NO:51], 0.1 μM each D4S2368 primers 1 [SEQ ID NO:56] and 2 [SEQ ID NO:57]], 0.1 μM each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 65 min. at

60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **32** which displays the amplified fragments of each locus. Lanes **1** to **4** contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253, D4S2368 and D20S481 and lane **5** displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

Example 33

Silver Detection of Multiplex Amplification of Loci D3S1539, D19S253, D13S317 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D3S1539, D19S253, D13S317 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 0.5 to 250 ng template, and 0.04 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Eight amplification primers were used in combination, including 0.5 μM each D3S1539 primers 1 [SEQ ID NO:7[and 2 [SEQ ID NO:49], 0.5 μM each D19S253 primers 1 [SEQ ID NO:50[and 2 (SEQ ID NO:51], 0.5 μM each D13S317 primers 1 (SEQ ID NO:3] and 2 [SEQ ID NO:4], 0.2 μM each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 68 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **33** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D3S1539, D19S253, D13S317 and D20S481 and lane **6** displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

Example 34

Silver Detection of Multiplex Amplification of Loci D10S1239, D9S930 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D9S930 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 0.5 to 250 ng template, and 0.03 U Taq DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Six amplification primers were used in combination, including 1.0 μM each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 4.0 μM each D9S930 primers 1 [SEQ ID NO:55] and 2 [SEQ ID NO:14], and 0.2 μM each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 68 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **34** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D10S1239, D9S930 and D20S481 and lane **6** displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

Example 35

Silver Detection of Multiplex Amplification of Loci D10S1239, D4S2368 and D20S481

In this example, a DNA template was amplified simultaneously at the individual loci, D10S1239, D4S2368 and D20S481 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1× STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$ and 200 μM each of dATP, dCTP, dGTP and dTTP) using 0.5 to 250 ng template, and 0.03 U Tag DNA Polymerase/μl. A Thermal Cycler 480 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 60° C. for 1 min., 70° C. for 1.5 min., followed by 1 cycle of 60° C. for 30 min.

Six amplification primers were used in combination, including 1.0 μM each D10S1239 primers 1 [SEQ ID NO:15] and 2 [SEQ ID NO:54], 0.2 μM each D4S2368 primers 1 [SEQ ID NO:56] and 2 [SEQ ID NO:57], and 0.2 μM each D20S481 primers 1 [SEQ ID NO:52] and 2 [SEQ ID NO:53].

Amplified products were separated by denaturing polyacrylamide gel electrophoresis on a 40 cm gel for 68 min. at 60 W and products were visualized by silver stain analysis according to the protocol of Bassam et al.(1991).

Reference is made to FIG. **35** which displays the amplified fragments of each locus. Lanes **1** to **5** contain DNA samples simultaneously co-amplified for the loci D10S1239, D4S2368 and D20S481 and lane **6** displays a sample without DNA template subjected to the same procedures, i.e., a negative control.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 61

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(i i) MOLECULE TYPE: Human Genomic DNA

(i i i) HYPOTHETICAL: no

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D7S820

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAACACTTGT CATAGTTTAG AACG 24

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D7S820

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTGAGGTATC AAAAACTCAG AGG 23

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D13S317

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACAGAAGTCT GGGATGTGGA 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D13S317

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCCCAAAAAG ACAGACAGAA 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D5S818

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

G G G T G A T T T T C C T C T T T G G T 2 0

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D5S818

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

T G A T T C C A A T C A T A G C C A C A 2 0

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D3S1539

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

T C T C T T T C C A T T A C T C T C T C C A T A G C 2 6

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D3S1539

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

A G T G C T G T T T T A G C T T C C A G G A 2 2

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D17S1298

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

G T A G G T C T T T T G G T T G C C A G T A T G 2 4

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D17S1298

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

T G T C A G T A A A C C T G T G A C C T G A G T 2 4

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D20S481

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

A A T G G T G A G A A A T G G G T T A T G A G T G C 2 6

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D20S481

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

T T T C C G G C T T T G T G T C A T A A A A C A G 2 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D9S930

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

T G G A C A A C A G A G T G A G A T G C 2 0

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D9S930

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

G C T A T G G G A A T T A C A A G C A G G A A 2 3

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D10S1239

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

C T T T G A A A T G G A C C C C T A G C T A A T G T 2 6

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D10S1239

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

C A C C C T G T C C C C A G C T A T C T G 2 1

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D14S118

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

C A G C T T G G G C A A C A T A G G G 1 9

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D14S118

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

C A A A C T C C T G A G G T C A A A C A A T C C 2 4

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D14S562

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

C T T G G A G G G T G G G G T G G C T A A 2 1

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D14S562

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

C G A A A T T T T G T T G C C T T G C T C T G G 2 4

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D14S548

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

C C T G G G C A A C A G A G T G A G A C T 2 1

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D14S548

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

A C C C A G C T T T A A C A G T T T G T G C T T 2 4

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D16S490

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:23:

G G G C G G A C A C A G A A T G T A A A A T C 2 3

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D16S490

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:24:

A A A C C C A A A T A G A T G A C A G G C A C A 2 4

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D16S753

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCACTCCAGG CTGAATGACA GAAC 24

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D16S753

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCAGTGCCGC CTATTTTTGT GAAT 24

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D17S1299

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ACCCTGATGA GATAGCACTT GAGC 24

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D17S1299

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CACTGTGTGG AGGTGTAGCA GAGA 24

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D16S539

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GGGGGTCTAA GAGCTTGTAA AAAG 24

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D16S539

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:30:

T G T G C A T C T G T A A G C A T G T A T C T A T C 2 6

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D22S683

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:31:

C G A A G G T T G C A T T G A G C C A A G A T 2 3

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D22S683

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:32:

T G G T G G A A A T G C C T C A T G T A G A A A 2 4

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMCSF1PO

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:33:

A A C C T G A G T C T G C C A A G G A C T A G C 2 4

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMCSF1PO

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:34:

T T C C A C A C A C C A C T G G C C A T C T T C 2 4

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMTPOX

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:35:

A C T G G C A C A G A A C A G G C A C T T A G G 2 4

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMTPOX

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:36:

G G A G G A A C T G G G A A C C A C A C A G G T 2 4

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMTHO1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:37:

A T T C A A A G G G T A T C T G G G C T C T G G 2 4

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMTHO1

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:38:

G T G G G C T G A A A A G C T C C C G A T T A T 2 4

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMvWFA31

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:39:

G A A A G C C C T A G T G G A T G A T A A G A A T A A T C 2 9

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMvWFA31

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGACAGATGA TAAATACATA GGATGGATGG 30

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMF13A01

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GAGGTTGCAC TCCAGCCTTT GCAA 24

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMF13A01

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TTCCTGAATC ATCCCAGAGC CACA 24

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMFESFPS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GCTGTTAATT CATGTAGGGA AGG 23

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMFESFPS

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTAGTCCCAG CTACTTGGCT ACTC 24

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20

-continued

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMBFXIII

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:45:

TGAGGTGGTG TACTACCATA 20

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMBFXIII

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GATCATGCCA TTGCACTCTA 20

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMLIPOL

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CTGACCAAGG ATAGTGGGAT ATAG 24

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMLIPOL

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGTAACTGAG CGAGACTGTG TCT 23

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D3S1539

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CCACCCTTTC AGCACCAG 18

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid

-continued

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D19S253

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:50:

A T A G A C A G A C A G A C G G A C T G 2 0

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D19S253

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:51:

G G G A G T G G A G A T T A C C C C T 1 9

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D20S481

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:52:

A A A G C T C T C T G A A G C A G G T G T 2 1

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D20S481

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:53:

C A G A T T G C A C T A G A A A G A G A G G A A 2 4

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D10S1239

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:54:

C A C C C T G T C C C C A G C T A T C T G G A 2 3

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: Nucleic Acid

-continued

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D9S930

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:55:

A G T T G A A T C T T G A G T C T C T C A G A G T C A 2 7

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D4S2368

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:56:

T G T A C T C A T T T T C C C G C A A T G A T G 2 4

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D4S2368

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:57:

T C A G A A A G T A G G G T C T G G G C T C T T 2 4

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D16S539

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:58:

T G T G C A T C T G T A A G C A T G T A T C T A T C A T 2 8

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMvWFA31

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:59:

G A A A G C C C T A G T G G A T G A T A A G A A T A A T C A G T 3 2

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: Nucleic Acid

-continued

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: HUMvWFA31

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGACAGATGA TAAATACATA GGATGGATGG ATA 33

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(v i i i) POSITION IN GENOME:
(B) MAP POSITION: D9S930

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GCTATGGGAA TTACAAGCAG GAAAC 25

What is claimed is:

1. A method of simultaneously determining the alleles present in at least four short tandem repeat loci from one or more DNA samples, comprising:

(a) obtaining at least one DNA sample to be analyzed,

(b) selecting a set of at least four short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein the at least four loci in the set are selected from the group of loci consisting of:
D3S1539, D4S2368, D5S818, D7S820, D9S930, D10S1239, D13S317, D14S118, D14S548, D14S562, D16S490, D16S539, D16S753, D17S1298, D17S1299, D19S253, D20S481, D22S683, HUMCSF1PO, HUMTPOX, HUMTH01, HUMF13A01, HUMBFXIII, HUMLIPOL, HUMvWFA31;

(c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

2. The method of claim **1**, wherein the set of at least four loci co-amplified therein is a set of four loci, wherein the set of four loci is selected from the group of sets of loci consisting of:

D3S1539, D7S820, D13S317, D5S818;
D17S1298, D7S820, D13S317, D5S818;
D20S481, D7S820, D13S317, D5S818;
D9S930, D7S820, D13S317, D5S818;
D10S1239, D7S820, D13S317, D5S818;
D14S118, D7S820, D13S317, D5S818;
D14S562, D7S820, D13S317, D5S818;
D14S548, D7S820, D13S317, D5S818;
D16S490, D7S820, D13S317, D5S818;
D17S1299, D7S820, D13S317, D5S818;
D16S539, D7S820, D13S317, D5S818;
D22S683, D7S820, D13S317, D5S818;
D16S753, D7S820, D13S317, D5S818;
D3S1539, D19S253, D13S317, D20S481;
D3S1539, D19S253, D4S2368, D20S481;
D10S1239, D9S930, D4S2368, D20S481; and
D16S539, D7S820, D13S317, HUMvWFA31.

3. The method of claim **1**, wherein the set of at least four loci co-amplified therein is a set of six loci, wherein the set of six loci is selected from the group of sets of loci consisting of:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS.

4. The method of claim **1**, wherein the set of at least four loci co-amplified therein is a set of seven loci, wherein the set is selected from the group of sets of loci consisting of:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII.

5. The method of claim **1**, wherein the set of at least four loci co-amplified therein is a set of at least eight loci, and wherein the set is selected from the group of sets of loci consisting of:

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31; and
D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII, HUMLIPOL.

6. The method of claim **1**, wherein the multiplex amplification reaction is done using at least four pair of primers flanking the at least four loci analyzed.

7. The method of claim **6**, additionally comprising the step of selecting pairs of primers for the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel electrophoresis.

8. The method of claim **6**, wherein at least one of each of the pairs of primers used in the multiplex amplification reaction has a sequence selected from one of the groups of sequences consisting of:

SEQ ID NO:1 and SEQ ID NO:2, when one of the loci in the set is D7S820;

SEQ ID NO:3 and SEQ ID NO:4, when one of the loci in the set is D13S317;

SEQ ID NO:5 and SEQ ID NO:6, when one of the loci in the set is D5S818;

SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:49, when one of the loci in the set is D3S1539;

SEQ ID NO:9, SEQ ID NO:10, when one of the loci in the set is D17S1298;

SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO:53, when one of the loci in the set is D20S481;

SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:55, SEQ ID NO:61, when one of the loci in the set is D9S930;

SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54, when one of the loci in the set is D10S1239;

SEQ ID NO:17, SEQ ID NO:18, when one of the loci in the set is D14S118;

SEQ ID NO:19, SEQ ID NO:20, when one of the loci in the set is D14S562;

SEQ ID NO:21, SEQ ID NO:22, when one of the loci in the set is D14S548;

SEQ ID NO:23, SEQ ID NO:24, when one of the loci in the set is D16S490;

SEQ ID NO:25, SEQ ID NO:26, when one of the loci in the set is D16S753;

SEQ ID NO:27, SEQ ID NO:28, when one of the loci in the set is D17S1299;

SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, when one of the loci in the set is D16S539;

SEQ ID NO:31, SEQ ID NO:32, when one of the loci in the set is D22S683;

SEQ ID NO:33, SEQ ID NO:34, when one of the loci in the set is HUMCSF1PO;

SEQ ID NO:35, SEQ ID NO:36, when one of the loci in the set is HUMTPOX;

SEQ ID NO:37, SEQ ID NO:38, when one of the loci in the set is HUMTH01;

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:59, SEQ ID NO:60 when one of the loci in the set is HUMvWFA31;

SEQ ID NO:41, SEQ ID NO:42, when one of the loci in the set is HUMF13A01;

SEQ ID NO:43, SEQ ID NO:44, when one of the loci in the set is HUMFESFPS;

SEQ ID NO:45, SEQ ID NO:46, when one of the loci in the set is HUMBFXIII;

SEQ ID NO:47, SEQ ID NO:48, when one of the loci in the set is HUMLIPOL;

SEQ ID NO:50, SEQ ID NO:51, when one of the loci in the set is D19S253; and

SEQ ID NO:56, SEQ ID NO:57, when one of the loci in the set is D4S2368.

9. The method of claim **6**, wherein the multiplex amplification reaction is a polymerase chain reaction.

10. The method of claim **1**, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

11. The method of claim **1**, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

12. The method of claim **11**, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

13. The method of claim **11**, wherein primers capable of binding to a region flanking each of the loci in the set are used in co-amplifying the loci, wherein at least one of the primers used in co-amplifying each locus has a fluorescent label covalently attached thereto such that the amplified alleles produced therefrom are fluorescently labeled, and wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescent analysis.

14. The method of claim **13**, wherein the fluorescent label is selected from the group of labels consisting of fluorescein and tetramethyl rhodamine.

15. The method of claim **1** wherein the at least one DNA sample to be analyzed is isolated from human tissue, wherein the human tissue is selected from the group of human tissue consisting of blood, semen, vaginal cells, hair, saliva, urine, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

16. A method of simultaneously determining the alleles present in three short tandem repeat loci from one or more DNA samples, comprising:

(a) obtaining at least one DNA sample to be analyzed,

(b) selecting a set of three short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein the set of three loci is selected from the group of sets of loci consisting of:
D3S1539, D19S253, D13S317;
D10S1239, D9S930, D20S481;
D10S1239, D4S2368, D20S481;
D10S1239, D9S930, D4S2368;
D16S539, D7S820, D13S317; and
D10S1239, D9S930, D13S317.

(c) co-amplifying the three loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

17. The method of claim **16**, wherein the multiplex amplification reaction is done using three pair of primers, wherein each pair of primers flanks one of the three short tandem repeat loci in the set of loci co-amplified in the reaction.

18. The method of claim **17**, wherein each of the three pair of primers used in the multiplex amplification reaction is designed to hybridize with an allele of a locus in the set of loci co-amplified in the reaction, wherein:

when D7S820 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2;

when D13S317 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4;

when D2OS481 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO:53;

when D9S930 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:55 and SEQ ID NO:61;

when D10S1239 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence

selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:44;

when D16S539 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:29 and SEQ ID NO:30; and

when D4S2368 is one of the loci in the set of loci co-amplified, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:56 and SEQ ID NO:57.

19. The method of claim **16**, wherein the multiplex amplification reaction is a polymerase chain reaction.

20. The method of claim **16**, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

21. The method of claim **16**, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

22. The method of claim **21**, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

23. The method of claim **21**, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescence analysis.

24. The method of claim **16** wherein the at least one DNA sample to be analyzed is isolated from human tissue, wherein the human tissue is selected from the group of human tissue consisting of blood, semen, vaginal cells, hair, saliva, urine, bone, buccal sample, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

25. A kit for simultaneously analyzing short tandem repeat sequences in at least three loci, comprising a container which has oligonucleotide primers for co-amplifying a set of at least three short tandem repeat loci, wherein the set of loci are selected from the sets of loci consisting of:

D3S1539, D19S253, D13S317;

D10S1239, D9S930, D20S481;

D10S1239, D4S2368, D20S481;

D10S1239, D9S930, D4S2368;

D16S539, D7S820, D13S317;

D10S1239, D9S930, D13S317;

D3S1539, D7S820, D13S317, D5S818;

D17S1298, D7S820, D13S317, D5S818;

D20S481, D7S820, D13S317, D5S818;

D9S930, D7S820, D13S317, D5S818;

D10S1239, D7S820, D13S317, D5S818;

D14S118, D7S820, D13S317, D5S818;

D14S562, D7S820, D13S317, D5S818;

D14S548, D7S820, D13S317, D5S818;

D16S490, D7S820, D13S317, D5S818;

D17S1299, D7S820, D13S317, D5S818;

D16S539, D7S820, D13S317, D5S818;

D22S683, D7S820, D13S317, D5S818;

D16S753, D7S820, D13S317, D5S818;

D3S1539, D19S253, D13S317, D20S481;

D3S1539, D19S253, D4S2368, D20S481;

D10S1239, D9S930, D4S2368, D20S481;

D16S539, D7S820, D13S317, HUMvWFA31;

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX;

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS;

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01;

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII;

D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31; and

D16S539, D7S820, D13S317, D5S818, HUMF13A01, HUMFESFPS, HUMBFXIII, HUMLIPOL.

26. The kit of claim **25**, wherein each of the oligonucleotide primers is designed to hybridize with an allele of one of the three loci in the set of loci selected, wherein:

when D7S820 is one of the loci in the set, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2;

when D13S317 is one of the loci in the set, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4;

when D5S818, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:6;

when D3S153, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:49;

when D17S1298, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:9 and SEQ ID NO:10;

when D20S481, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:52, SEQ ID NO:53;

when D9S930, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:55, SEQ ID NO:61;

when D10S1239, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:54;

when D14S118, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18;

when D14S562, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20;

when D14S548, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22;

when D16S490, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:24;

when D16S753, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:26;

when D17S1299, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28;

when D16S539, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58;

when D22S683, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:31, SEQ ID NO:32;

when HUMCSF1PO, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:33, SEQ ID NO:34;

when HUMTPOX, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:35; SEQ ID NO:36;

when HUMTH01, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:37, SEQ ID NO:38;

when HUMvWFA31, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:60;

when HUMF13A01, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42;

when HUMFESFPS, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44;

when HUMBFXIII, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46;

when HUMLIPOL, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:47, SEQ ID NO:48;

when D19S253, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:50, SEQ ID NO:51; and

when D4S2368, at least one of the primers has a sequence selected from the group consisting of SEQ ID NO:56, SEQ ID NO:57.

27. The kit of claim **25**, further comprising a container having reagents for at least one multiplex amplification reaction.

28. The kit of claim **25**, further comprising a container having an allelic ladder.

29. The kit of claim **28**, wherein each rung of the allelic ladder and at least one oligonucleotide primer for each of the loci in the set each have a label covalently attached thereto.

30. The kit of claim **29**, wherein the label is a fluorescent label.

31. The kit of claim **30**, wherein at least one of the oligonucleotide primers has a different fluorescent label covalently attached thereto than some of the other primer pairs in the container.

32. A method of simultaneously determining the alleles present in at least four short tandem repeat loci from one or more DNA samples, comprising:

(a) obtaining at least one DNA sample to be analyzed,

(b) selecting a set of at least four short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein three of the loci in the set are D7S820, D13S317, and D5S818;

(c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

33. The method of claim **32**, wherein the multiplex amplification reaction is done using at least four pair of primers flanking the at least four loci analyzed.

34. The method of claim **33**, additionally comprising the step of selecting pairs of primers for the multiplex amplification reaction which produce alleles from each locus that do not overlap the alleles of the other loci in the set co-amplified therein, when the alleles are separated by gel electrophoresis.

35. The method of claim **32**, wherein the multiplex amplification reaction is a polymerase chain reaction.

36. The method of claim **32**, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

37. The method of claim **32**, wherein the amplified alleles are evaluated using polyacrylamide gel electrophoresis to separate the alleles, forming a polyacrylamide gel of separated alleles.

38. The method of claim **37**, wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with silver stain analysis.

39. The method of claim **37**, wherein primers capable of binding to a region flanking each of the loci in the set are used in co-amplifying the loci, wherein at least one of the primers used in co-amplifying each locus has a fluorescent label covalently attached thereto such that the amplified alleles produced therefrom are fluorescently labeled, and wherein the separated alleles in the polyacrylamide gel are determined by visualizing the alleles with fluorescence analysis.

40. The method of claim **39**, wherein the fluorescent label is selected from the group of labels consisting of fluorescein and tetramethyl rhodamine.

41. The method of claim **6**, wherein one of each of at the least four pair of primers used in the multiplex amplification reaction has a fluorescent label covalently attached thereto.

42. The method of claim **41**, wherein at least four of the primers used in the multiplex amplification reaction have the same fluorescent label covalently attached thereto.

43. The kit of claim **30**, wherein at least four of the oligonucleotide primers have the same fluorescent label covalently attached thereto.

* * * * *

EXHIBIT 7


US006221598B1

(12) **United States Patent**
Schumm et al.

(10) **Patent No.: US 6,221,598 B1**
(45) **Date of Patent: *Apr. 24, 2001**

(54) **MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI**

(75) Inventors: **James W. Schumm**; **Cynthia J. Sprecher**, both of Madison; **Ann M. Lins**, Lodi, all of WI (US)

(73) Assignee: **Promega Corporation**, Madison, WI (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/327,229**

(22) Filed: **Jun. 7, 1999**

Related U.S. Application Data

(63) Continuation of application No. 08/316,544, filed on Sep. 30, 1994, now abandoned.

(51) **Int. Cl.**[7] **C12Q 1/70**; C07H 21/04

(52) **U.S. Cl.** **435/6**; 435/91.1; 435/91.2; 536/23.1; 536/24.31; 536/24.33

(58) **Field of Search** 435/6, 91.2, 91.1; 536/24.33, 24.31, 23.1

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Attachment #1, Sequence Alignments for SEQ ID No: 1, 2, 7, 8, 11, 15, 16, 19, 20, 27, and 28.*
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Primary Examiner—W. Gary Jones
Assistant Examiner—Jeanine Enewold
(74) *Attorney, Agent, or Firm*—Michael Best & Friedrich LLP; Grady J. Frenchick; Karen B. King

(57) **ABSTRACT**

The present invention is directed to the simultaneous amplification of multiple distinct genetic loci using PCR or other amplification systems to determine in one reaction the alleles of each locus contained within the multiplex.

43 Claims, 7 Drawing Sheets

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FIG. 1

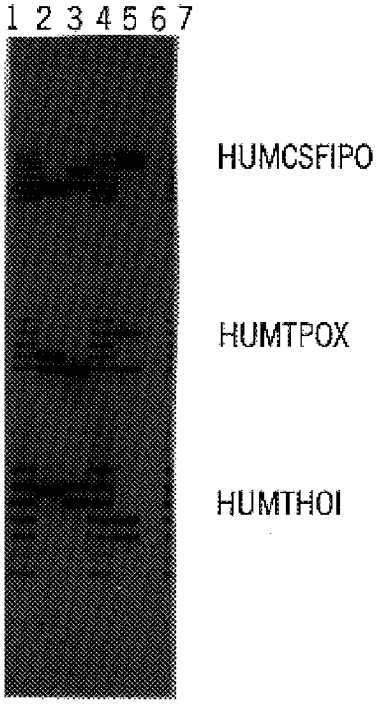


FIG. 2

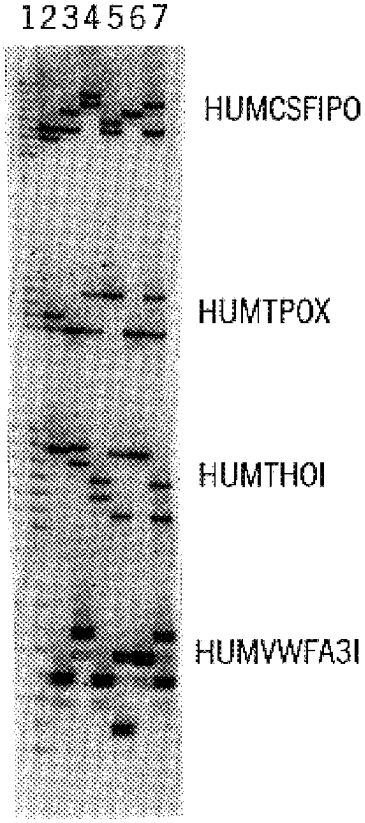


FIG. 3

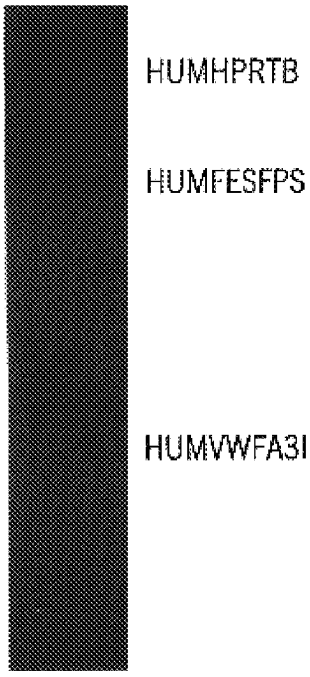


FIG. 4

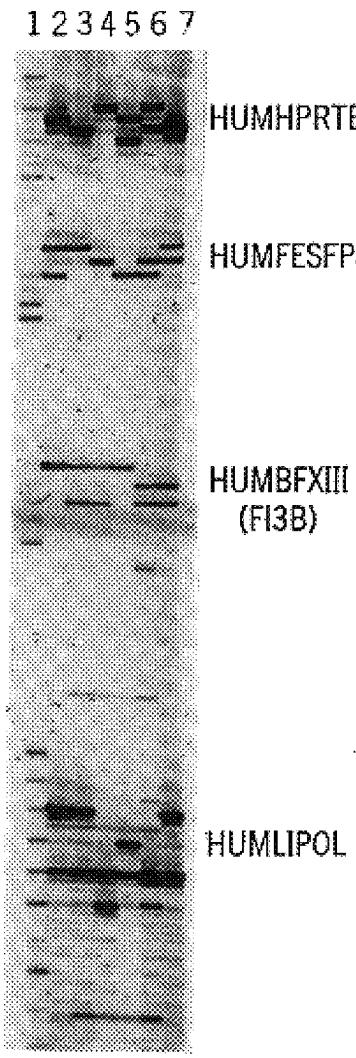


FIG. 5

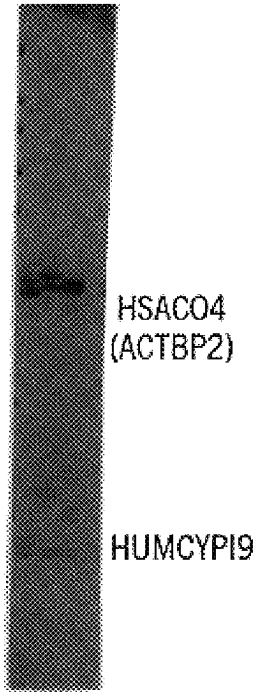


FIG. 6

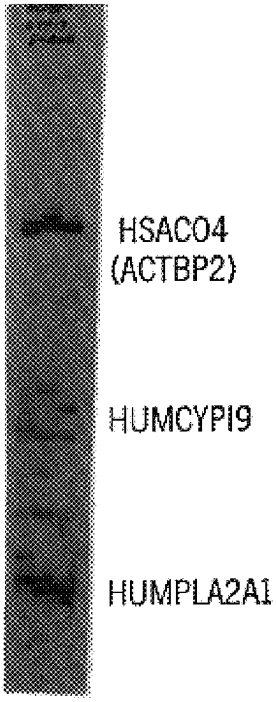


FIG. 7

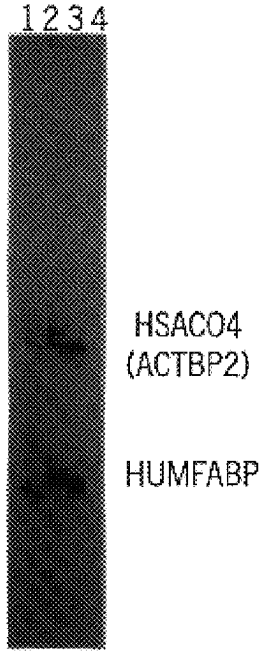


FIG. 8

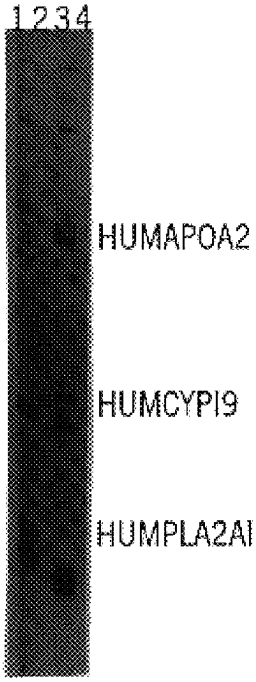


FIG. 9

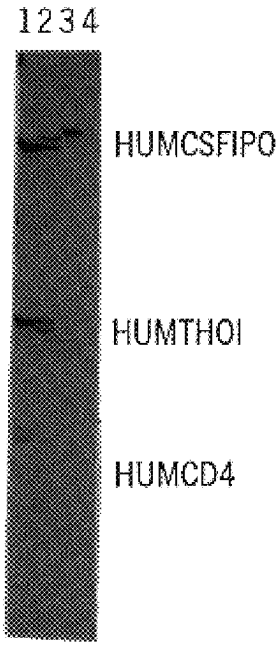


FIG. 10

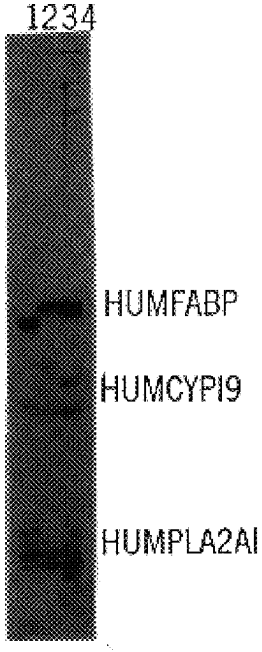


FIG. 11

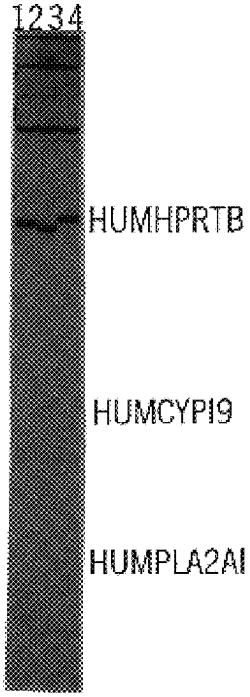


FIG. 12

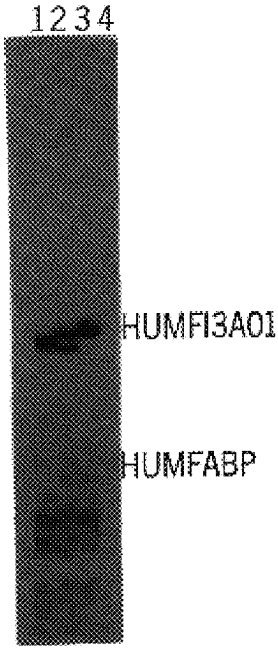


FIG. 13

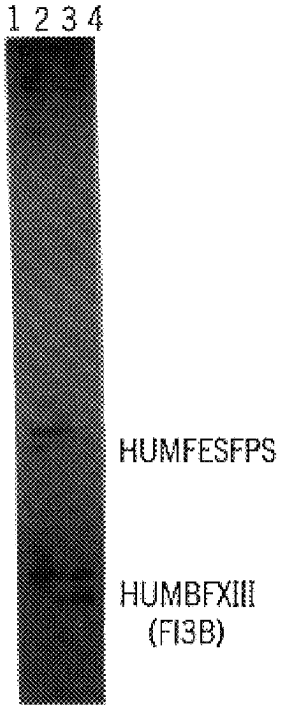


FIG. 14

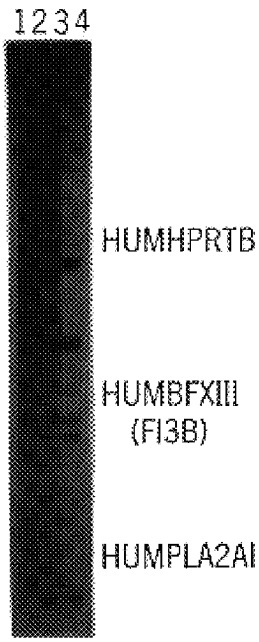


FIG. 15

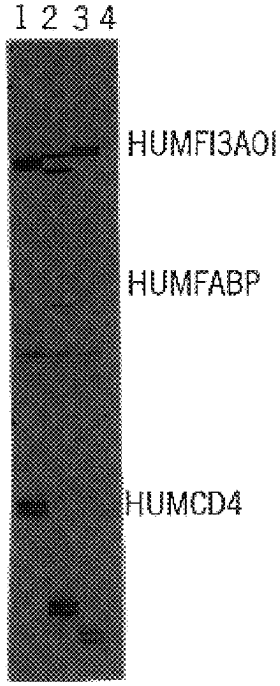


FIG. 16

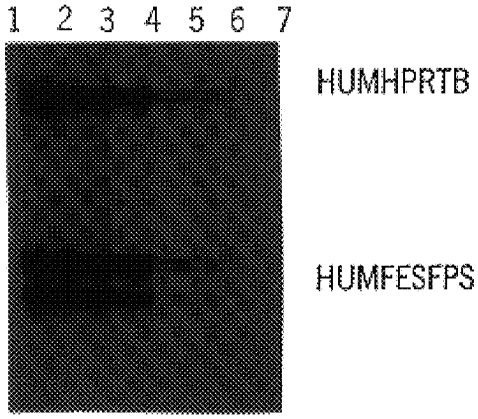


FIG. 17

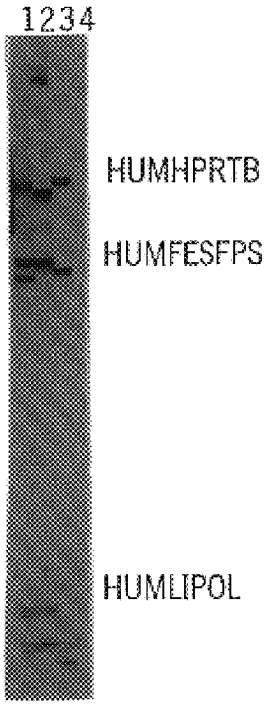


FIG. 18

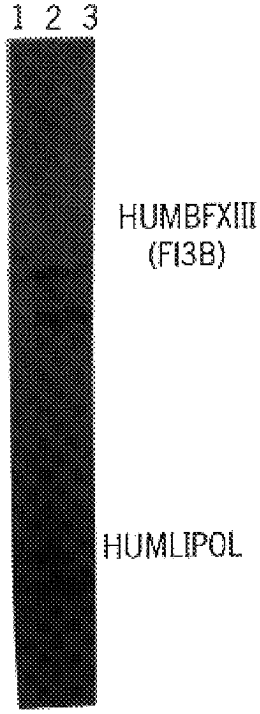


FIG. 19

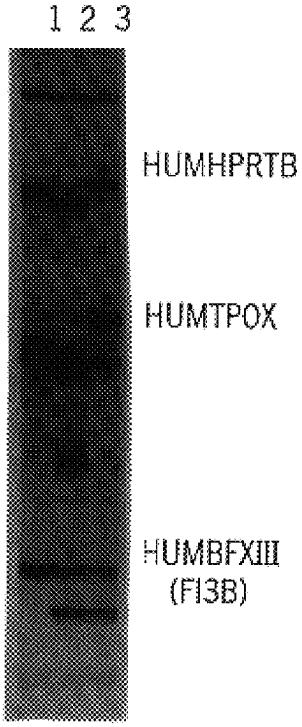


FIG. 20

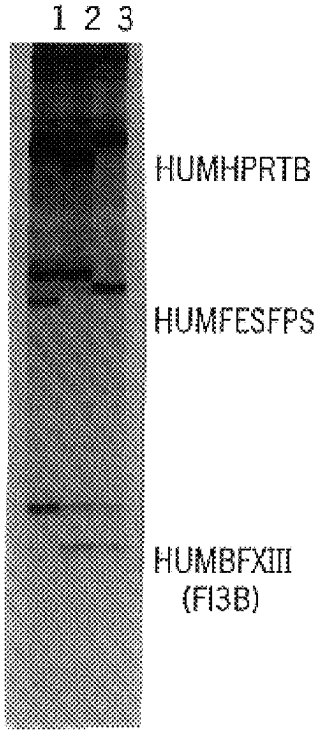


FIG. 21

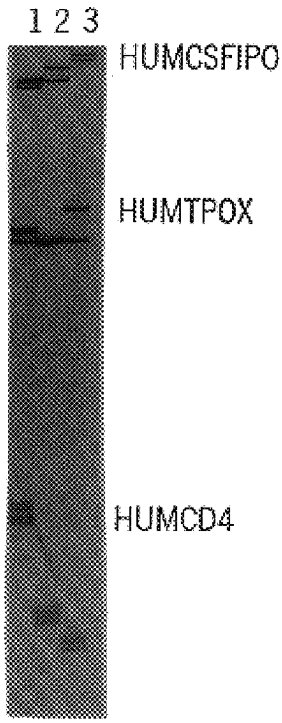


FIG. 22

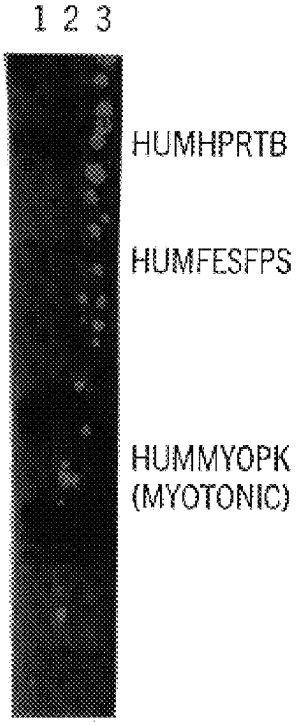


FIG. 23

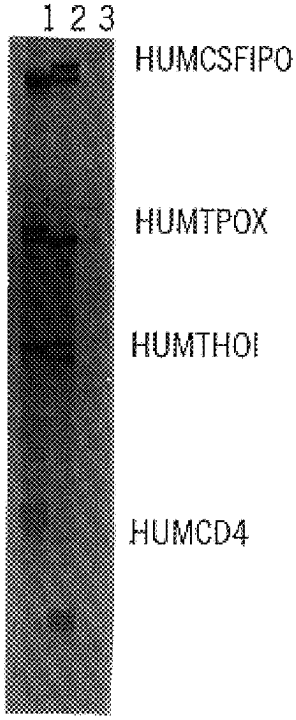


FIG. 24

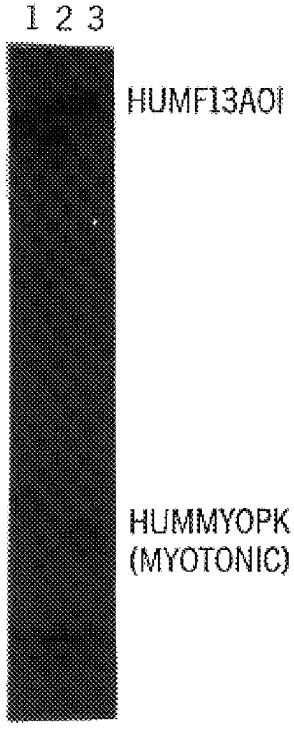


FIG. 25

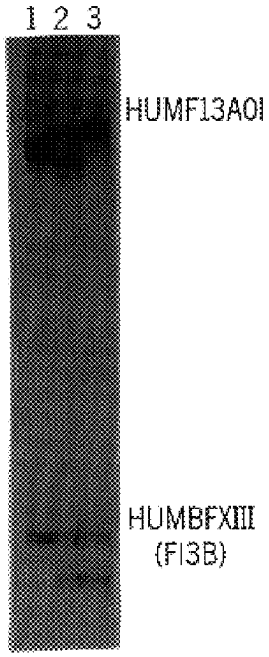


FIG. 26

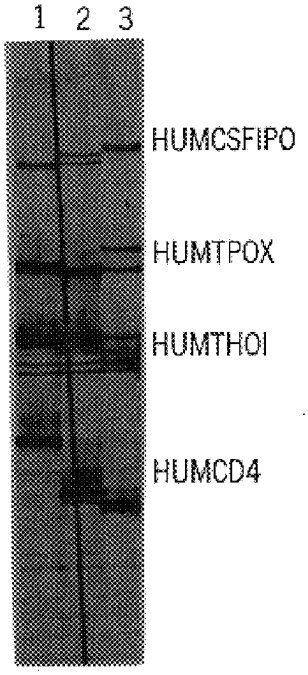


FIG. 27

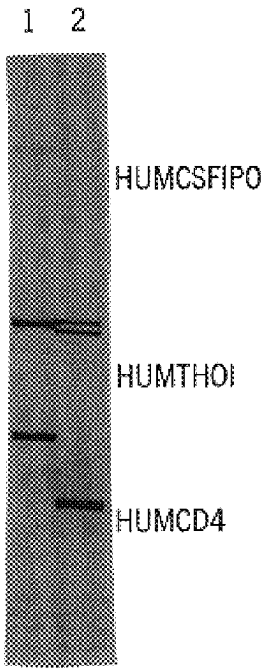


FIG. 28

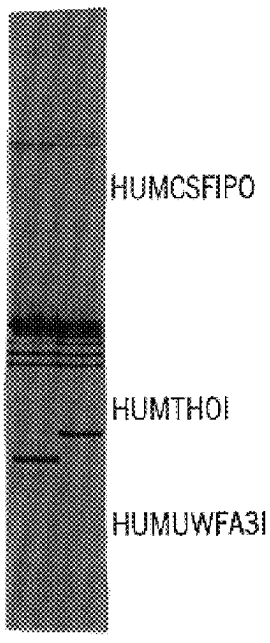


FIG. 29

FIG. 30

FIG. 31

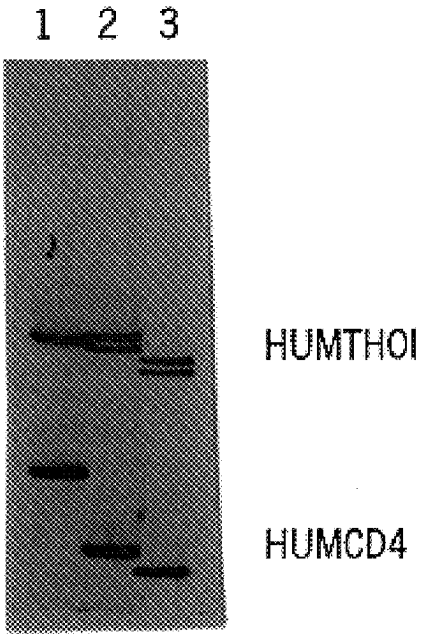
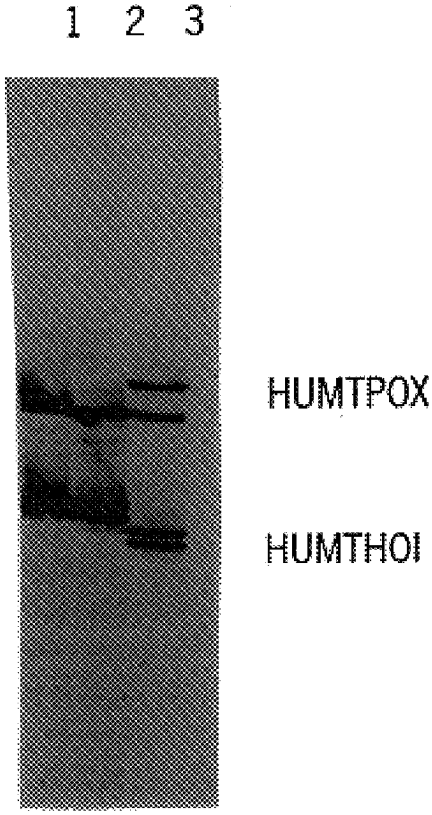


FIG. 32



MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. patent application Ser. No. 08/316,544, filed Sep. 30, 1994, now abandoned.

FIELD OF THE INVENTION

The present invention is generally directed to the detection of genetic markers in a genomic system. The present invention is more specifically directed to the simultaneous amplification of multiple distinct polymorphic genetic loci using the polymerase chain reaction or other amplification systems to determine in one reaction the alleles of each locus contained within the multiplex system.

CITED REFERENCES

A full bibliographic citation of the references cited in this application can be found in the section preceding the claims.

DESCRIPTION OF THE PRIOR ART

In recent years, the discovery and development of polymorphic short tandem repeats (STRs) as genetic markers has stimulated progress in the development of linkage maps, the identification and characterization of diseased genes, and the simplification and precision of DNA typing.

Many loci, at least in the human genome, contain a polymorphic STR region. STR loci consist of short, repetitive sequence elements of 3 to 7 base pairs in length. It is estimated that there are 2,000,000 expected trimeric and tetrameric STRs present as frequently as once every 15 kilobases (kb) in the human genome (Edwards et al. 1991; Beckmann and Weber 1992). Nearly half of the STR loci studied by Edwards et al. (1991) are polymorphic, which provides a rich source of genetic markers. Variation in the number of repeat units at a particular locus is responsible for the observed polymorphism reminiscent of VNTR loci (Nakamura et al. 1987) and minisatellite loci (Jeffreys et al. 1985), which contain longer repeat units, and microsatellite or dinucleotide repeat loci (Litt and Luty 1989, Tautz 1989, Weber and May 1989, Beckmann and Weber 1992).

Polymorphic STR loci are extremely useful markers for human identification, paternity testing and genetic mapping. STR loci may be amplified via the polymerase chain reaction (PCR) by employing specific primer sequences identified in the regions flanking the tandem repeat.

Alleles of these loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another following electrophoretic separation by any suitable detection method including radioactivity, fluorescence, silver stain, and color.

To minimize labor, materials and analysis time, it is desirable to analyze multiple loci and/or more samples simultaneously. One approach for reaching this goal involves amplification of multiple loci simultaneously in a single reaction. Such "multiplex" amplifications have been described extensively in the literature. Multiplex amplification sets have been extensively developed for analysis of genes related to human genetic diseases such as Duchenne Muscular Dystrophy (Chamberlain et al. 1988, Chamberlain et al. 1989, Beggs et al. 1990, Clemens et al. 1991, Schwartz et al. 1992, Covone et al. 1992), Lesch-Nyhan Syndrome (Gibbs et al. 1990), Cystic Fibrosis (Estivill et al. 1991, Fortina et al. 1992, Ferrie et al. 1992, Morral and Estivill, 1992), and Retinoblasma (Lohmann et al. 1992). Multiplex amplification of polymorphic microsatellite markers (Clemens et al. 1991, Schwartz et al. 1992, Huang et al. 1992) and even STR markers (Edwards et al. 1992, Kimpton et al. 1993, Hammond et al. 1994) have been described.

These amplified products are generally separated by one of several methods of electrophoresis known to those skilled in the art. Several well-known methods of detection of the amplified products have also been described. While ethidium bromide staining of amplified fragments is employed in some cases, in others it is preferred to use methods which label only one of the two strands of the amplified material. Examples of this include radioactive or fluorescent labeling of one of the two primers prior to the amplification of a locus. One of the more sophisticated approaches to detection is the use of different fluorescent labels to allow detection of amplified materials representing different loci, but existing in the same space following electrophoresis. The products of the different loci are differentiated with the use of filters, which allow visualization of one fluorescent label at a time.

Reference is made to International Publications WO 93/18177 and WO 93/18178 to Fortina et al., which are directed to methods and kits for diagnosing diseases such as Cystic Fibrosis and β-thalassemia, respectively, using an allele-specific multiplex polymerase chain reaction system. According to Fortina et al., multiplex PCR has also been used for simultaneous amplification of multiple target sequences, permitting mutant allele scanning using two lanes of an agarose gel.

Ballabio et al. (1991), disclose a single-tube, multiplex allele specific PCR test using two different dye-tagged fluorescent primers for detection of the ▲F508 cystic fibrosis mutation.

While there are multiplex amplification procedures for specific loci, the use of multiplex amplification procedures is greatly desired for the detection of alleles in other types of loci such as specific STR loci.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a method for the simultaneous amplification of multiple distinct polymorphic STR loci using PCR or other amplification systems to determine, in one reaction, the alleles of each locus contained within the multiplex. These combinations of specific loci into multiplexes have not been heretofore shown.

It is also an object of the present invention to provide a method and a kit specific for multiplex amplifications comprising specified loci.

These and other objects are addressed by the present invention which is directed to a method of simultaneously analyzing or determining the alleles present at each individual locus of each multiplex. This method comprises the steps of (1) obtaining at least one DNA sample to be analyzed, wherein the DNA sample has at least two loci which can be amplified together; (2) amplifying the STR sequences in the DNA sample; and (3) detecting the amplified materials in a fashion which reveals the polymorphic nature of the systems employed.

The present invention is also directed to a method of simultaneously analyzing multiple STR sequences wherein at least one of the loci is selected from the group consisting of: HUMCSF1PO, HUMTPOX, HUMVWFA31, HUMFESFPS, HUMBFXIII (F13B), HUMLIPOL,

HSAC04 (ACTBP2), HUMCYP19, HUMPLA2A1, HUMAPOA2, HUMCD4, HUMF13A01 and HUMMYOPK (Myotonic).

Specifically, the present invention is directed to a method of simultaneously analyzing multiple STR sequences in the following groups of loci: HUMTH01 and HUMCSF1PO; HUMTH01 and HUMCD4; HUMTH01 and HUMTPOX; HUMF13A01 and HUMFABP; HUMF13A01 and HUMMYOPK (Myotonic); HUMF13A01 and HUMBFXIII (F13B); HUMBFXIII (F13B) and HUMFESFPS; HUMBFXIII (F13B) and HUMLIPOL; HUMHPRTB and HUMFESFPS; HSAC04 (ACTBP2) and HUMCYP19; HUMCSF1PO, HUMTPOX and HUMTH01; HUMHPRTB, HUMFESFPS and HUMVWFA31; HSAC04 (ACTBP2), HUMCYP19 and HUMPLA2A1; HSAC04 (ACTBP2) and HUMFABP; HUMAPOA2, HUMCYP19 and HUMPLA2A1; HUMCD4, HUMCSF1PO and HUMTH01; HUMCYP19, HUMFABP and HUMPLA2A1; HUMCYP19, HUMHPRTB and HUMPLA2A1; HUMF13A01, HUMFABP and HUMCD4; HUMHPRTB, HUMFESFPS and HUMLIPOL; HUMF13A01, HUMFABP and HUMCD4; HUMHPRTB, HUMBFXIII (F13B) and HUMPLA2A1; HUMHPRTB, HUMBFXIII (F13B) and HUMTPOX; HUMHPRTB, HUMBFXIII (F13B) and HUMFESFPS; HUMCSF1PO, HUMTPOX and HUMCD4; HUMHPRTB, HUMFESFPS and HUMMYOPK (Myotonic); HUMCSF1PO, HUMTH01 and HUMCD4; HUMCSF1PO, HUMTH01 and HUMVWFA31; HUMHPRTB, HUMBFXIII (F13B) and HUMLIPOL; HUMCSF1PO, HUMTPOX, HUMTH01 and HUMVWFA31; HUMHPRTB, HUMFESFPS, HUMBFXIII (F13B) and HUMLIPOL; HUMCSF1PO, HUMTPOX, HUMTH01 and HUMCD4; and HUMCSF1PO, HUMTH01, HUMTPOX and HUMCD4.

The present invention provides a high throughput method for the detection and analysis of polymorphic genetic markers using specific combinations of loci and specified conditions. By selection of the appropriate detection method, the process can be used in laboratories which have only a power supply and a standard apparatus for polyacrylamide gel electrophoresis or those which have the latest in equipment for fluorescent gel scanning, e.g., FluorImager™ −575 (Molecular Dynamics, Sunnyvale, Calif.). Thus, the process of the present invention is adaptable for a variety of uses and laboratories.

The approach as specified in the present invention produces a savings in time, labor and materials in the analysis of loci contained within the multiplexes. The process of the present invention includes all the requisite primers, allowing between two and four or more loci to be amplified together in one amplification tube instead of amplifying each locus independently.

The present invention has specific use in the field of forensic analysis, paternity determination, monitoring of bone marrow transplantation, linkage mapping, and detection of genetic diseases and cancers.

These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention and the attached drawings and photographs.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a photograph illustrating the simultaneous amplification of three loci: HUMCSF1PO, HUMTPOX and HUMTH01, with the amplified products of each locus shown migrating next to the corresponding allelic ladder for ease of interpretation in Example 1.

FIG. **2** is a computer image showing the fluorescent detection of multiplex amplification of the loci HUMCSF1PO, HUMTPOX, HUMTH01 and HUMVWFA31 as detected with a FluorImager™ −575 (Molecular Dynamics, Sunnyvale, Calif.) in Example 2.

FIG. **3** is a photograph showing the silver stain detection of the multiplex amplification in Example 3.

FIG. **4** is a computer image showing the fluorescent detection of multiplex amplification in Example 4.

FIG. **5** is a photograph showing the silver stain detection of the multiplex amplification in Example 5.

FIG. **6** is a photograph showing the silver stain detection of the multiplex amplification in Example 6.

FIG. **7** is a photograph showing the silver stain detection of the multiplex amplification in Example 7.

FIG. **8** is a photograph showing the silver stain detection of the multiplex amplification in Example 8.

FIG. **9** is a photograph showing the silver stain detection of the multiplex amplification in Example 9.

FIG. **10** is a photograph showing the silver stain detection of the multiplex amplification in Example 10.

FIG. **11** is a photograph showing the silver stain detection of the multiplex amplification in Example 11.

FIG. **12** is a photograph showing the silver stain detection of the multiplex amplification in Example 12.

FIG. **13** is a photograph showing the silver stain detection of the multiplex amplification in Example 13.

FIG. **14** is a photograph showing the silver stain detection of the multiplex amplification in Example 14.

FIG. **15** is a photograph showing the silver stain detection of the multiplex amplification in Example 15.

FIG. **16** is a photograph showing the silver stain detection of the multiplex amplification in Example 16.

FIG. **17** is a photograph showing the silver stain detection of the multiplex amplification in Example 17.

FIG. **18** is a photograph showing the silver stain detection of the multiplex amplification in Example 18.

FIG. **19** is a photograph showing the silver stain detection of the multiplex amplification in Example 19.

FIG. **20** is a photograph showing the silver stain detection of the multiplex amplification in Example 20.

FIG. **21** is a photograph showing the silver stain detection of the multiplex amplification in Example 21.

FIG. **22** is a photograph showing the silver stain detection of the multiplex amplification in Example 22.

FIG. **23** is a photograph showing the silver stain detection of the multiplex amplification in Example 23.

FIG. **24** is a photograph showing the silver stained detection of the multiplex amplification in example 24.

FIG. **25** is a photograph showing the silver stain detection of the multiplex amplification in Example 25.

FIG. **26** is a photograph of a computer image showing the fluorescent detection of the multiplex amplification in Example 26.

FIG. **27** is a photograph of a computer image showing the fluorescent detection of the multiplex amplification in Example 27.

FIG. **28** is a photograph of a computer image showing the fluorescent detection of the multiplex amplification in Example 28.

FIG. **29** is a photograph of a computer image showing the fluorescent detection of the multiplex amplification in Example 29.

FIG. **30** is a photograph of a computer image showing the fluorescent detection of the multiplex amplification in Example 30.

FIG. **31** is a photograph of a computer image showing the fluorescent detection of the multiplex amplification in Example 31.

FIG. **32** is a photograph of a computer image showing the fluorescent detection of the multiplex amplification in Example 32.

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are intended to assist in providing a clear and consistent understanding of the scope and detail of the terms:

Allelic ladder: a standard size marker consisting of amplified alleles from the locus.

Allele: a genetic variation associated with a segment of DNA, i.e., one of two or more alternate forms of a DNA sequence occupying the same locus.

Biochemical nomenclature: standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and cytosine (C). Corresponding nucleotides are, for example, deoxyguanosine-5'-triphosphate (dGTP).

DNA polymorphism: the condition in which two or more different nucleotide sequences coexist in the same interbreeding population in a DNA sequence.

Locus (or genetic locus): a specific position on a chromosome. Alleles of a locus are located at identical sites on homologous chromosomes.

Locus-specific primer: a primer that specifically hybridizes with a portion of the stated locus or its complementary strand, at least for one allele of the locus, and does not hybridize efficiently with other DNA sequences under the conditions used in the amplification method.

Polymerase chain reaction (PCR): a technique in which cycles of denaturation, annealing with primer, and extension with DNA polymerase are used to amplify the number of copies of a target DNA sequence by $>10^6$ times. The polymerase chain reaction process for amplifying nucleic acid is covered by U. S. Pat. Nos. 4,683,195 and 4,683,202, which are incorporated herein by reference for a description of the process.

Polymorphism information content (PIC): a measure of the amount of polymorphism present at a locus (*Botstein* et al., 1980). PIC values range from 0 to 1.0, with higher values indicating greater degrees of polymorphism. This measure generally displays smaller values than the other commonly used measure, i.e., heterozygosity. For markers that are highly informative (heterozygosities exceeding about 70%), the difference between heterozygosity and PIC is slight.

Primary reaction: initial reaction using the purified human genomic DNA as template for the PCR.

Primers: two single-stranded oligonucleotides or DNA fragments which hybridize with opposing strands of a locus such that the 3' termini of the primers are in closest proximity.

Primer pair: two primers including primer 1 that hybridizes to a single strand at one end of the DNA sequence to be amplified and primer 2 that hybridizes with the other end on the complementary strand of the DNA sequence to be amplified.

Primer site: the area of the target DNA to which a primer hybridizes.

Secondary reaction: reamplification with the same or different primer pair using a dilution of the primary reaction as template for the PCR.

Construction of the Multiplex System

Prior to constructing the multiplex system, an appropriate set of loci, primers, and amplification protocols must be selected such that amplification generates fragments such that alleles of the various loci do not overlap in size or, when such overlap occurs, fragments representing different loci are detectable by separate means. In addition, the selected loci must be compatible for use with a single amplification protocol. The specific combinations of loci described herein are unique in this application. Combinations of loci may be rejected for either of these reasons, or because, in combination, one or more of the loci do not produce adequate product yield, or fragments which do not represent authentic alleles are produced in this reaction.

Successful combinations are generated by trial and error of locus combinations and by adjustment of primer concentrations to identify an equilibrium in which all included loci may be amplified.

Of particular importance in the multiplex system is the size range of amplified alleles produced from the individual loci which will be analyzed together. For ease of analysis with current technologies, systems which can be detected by amplification of fragments smaller than 500 bases were preferably selected.

The following multiplex combinations have been developed and are considered ideal combinations for use in the present system:

1. HUMTH01 and HUMCSF1PO;
2. HUMTH01 and HUMCD4;
3. HUMTH01 and HUMTPOX;
4. HUMF13A01 and HUMFABP;
5. HUMF13A01 and HUMMYOPK (Myotonic);
6. HUMF13A01 and HUMBFXIII (F13B);
7. HUMBFXIII (F13B) and HUMFESFPS;
8. HUMBFXIII (F13B) and HUMLIPOL;
9. HUMHPRTB and HUMFESFPS;
10. HSAC04 (ACTBP2) and HUMCYP19;
11. HSAC04 (ACTBP2) and HUMFABP;
12. HUMCSF1PO, HUMTPOX and HUMTH01;
13. HUMHPRTB, HUMFESFPS and HUMVWFA31;
14. HSAC04 (ACTBP2), HUMCYP19 and HUMPLA2A1;
15. HUMAPOA2, HUMCYP19 and HUMPLA2A1;
16. HUMCD4, HUMCSF1PO and HUMTH01;
17. HUMCYP19, HUMFABP and HUMPLA2A1;
18. HUMCYP19, HUMHPRTB and HUMPLA2A1;
19. HUMF13A01, HUMFABP and HUMCD4;
20. HUMHPRTB, HUMFESFPS and HUMLIPOL;
21. HUMF13A01, HUMFABP and HUMCD4;
22. HUMHPRTB, HUMBFXIII (F13B) and HUMPLA2A1;
23. HUMHPRTB, HUMBFXIII (F13B) and HUMTPOX;
24. HUMHPRTB, HUMBFXIII (F13B) and HUMFESFPS;
25. HUMCSF1PO, HUMTPOX and HUMCD4;
26. HUMHPRTB, HUMFESFPS and HUMMYOPK (Myotonic);
27. HUMCSF1PO, HUMTH01 and HUMCD4;
28. HUMCSF1PO, HUMTH01 and HUMVWFA31;
29. HUMHPRTB, HUMBFXIII (F13B) and HUMLIPOL;
30. HUMCSF1PO, HUMTPOX, HUMTH01 and HUMVWFA31;
31. HUMHPRTB, HUMFESFPS, HUMBFXIII (F13B) and HUMLIPOL;
32. HUMCSF1PO, HUMTPOX, HUMTH01 and HUMCD4; and
33. HUMCSF1PO, HUMTH01, HUMTPOX and HUMCD4.

The primers must also be designed so that the size of the resulting amplification products differ in length, thereby facilitating assignment of alleles to individual loci during detection. Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplification at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex. The synthesis of the primers is conducted by procedures known to those skilled in the art.

Using Multiplexes of Two Loci to Develop Multiplexes Using More than Two Loci

Once a multiplex containing two loci is developed, it may be used as a core to create multiplexes containing more than two loci. New combinations are created including the first two loci. For example, the core multiplex containing loci HUMTH01 and HUMCSF1PO was used to generate derivative multiplexes of HUMTH01, HUMCSF1PO, and HUMTPOX; HUMTH01, HUMCSF1PO, and HUMCD4; HUMTH01, HUMCSF1PO, and HUMVWFA31; HUMTH01, HUMCSF1PO, HUMVWFA31, and HUMTPOX; and HUMTH01, HUMCSF1PO, HUMCD4, and HUMTPOX. Many other derivative multiplexes can be generated based upon a working multiplex. The derivative multiplexes are, in some sense, routine extensions of the core multiplex.

Preparation of Genomic DNA

All methods of DNA preparation which are compatible with the amplification process for a single locus should be appropriate for multiplex amplification. Many examples of preparation methods have been described in the literature (Patel et al. 1984, Gill et al. 1985). DNA concentrations are measured fluorometrically (Brunk et al. 1979).

Amplification of DNA

Human genomic DNA samples are subjected to PCR amplification using primers and thermocycling conditions specific for each locus. Reference is made to Table 1 for details of the primer sequences. The amplification protocol specific to each multiplex is listed in the specific examples.

Designation	Primer sequences	Sequence ID Number
HSAC04	primer 1: ACA TCT CCC CTA CCG CTA TA	1
(ACTBP2)	primer 2: AAT CTG GGC GAC AAG AGT GA	2
HUMAPOA2	primer 1: GGA GCA GTC CTA GGG CCG CGC CGT	3
(APOCIII)	primer 2: GTG ACA GAG GGA GAC TCC ATT AAA	4
HUMCSF1PO	primer 1: AAC CTG AGT CTG CCA AGG ACT AGC	5
	primer 2: TTC CAC ACA CCA CTG GCC ATC TTC	6
HUMCYP19	primer 1: GCA GGT ACT TAG TTA GCT AC	7
(CYARP450)	primer 2: TTA CAG TGA GCC AAG GTC GT	8
HUMCD4	primer 1: CCA GGA AGT TGA GGC TGC AGT GAA	9
	primer 2: TTG GAG TCG CAA GCT GAA CTA GCG	10
HUMF13A01	primer 1: GAG GTT GCA CTC CAG CCT TTG CAA	11
	primer 2: TTC CTG AAT CAT CCC AGA GCC ACA	12
HUMBFXIII	primer 1: TGA GGT GGT GTA CTA CCA TA	13
(F13B)	primer 2: GAT CAT GCC ATT GCA CTC TA	14
HUMFABP	primer 1: GTA GTA TCA GTT TCA TAG GGT CAC C	15
	primer 2: CAG TTC GTT TCC ATT GTC TGT CCG	16
HUMFESFPS	primer 1: GCT GTT AAT TCA TGT AGG GAA GGC	17
	primer 2: GTA GTC CCA GCT ACT TGG CTA CTC	18
HUMHPRTB	primer 1: ATG CCA CAG ATA ATA CAC ATC CCC	19
(HPRT-1)	primer 2: CTC TCC AGA ATA GTT AGA TGT AGG	20
HUMMYOPK	primer 1: GCT CGA AGG GTC CTT GTA GCC GGG	21
Myotonic	primer 2: GAT AGG TGG GGG TGC GTG GAG GAT	22
HUMLIPOL	primer 1: CTG ACC AAG GAT AGT GGG ATA TAG	23
	primer 2: GGT AAC TGA GCG AGA CTG TGT CT	24
HUMPLA2A1	primer 1: GGT TGT AAG CTC CAT GAG GTT AGA	25

-continued

Designation	Primer sequences	Sequence ID Number
(PLA-AZ)	primer 2: TTG AGC ACT TAC TAT GTG CCA GGC T	26
HUMTH01	primer 1: GTG GGC TGA AAA GCT CCC GAT TAT	27
	primer 2: ATT CAA AGG GTA TCT GGG CTC TGG	28
HUMTPOX	primer 1: ACT GGC ACA GAA CAG GCA CTT AGG	29
	primer 2: GGA GGA ACT GGG AAC CAC ACA GGT	30
HUMVWFA31	primer 1: GA AAG CCC TAG TGG ATG ATA AGA ATA ATC	31
	primer 2: GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG	32

Reference is made to the examples below for additional details of the specific procedure relating to each multiplex. The locus-specific primers include a number of nucleotides which, under the conditions used in the hybridization, are sufficient to hybridize with an allele of the locus to be amplified and to be essentially free from amplification of alleles of other loci. Reference is made to U. S. Pat. 5,192,659 to Simons, which is incorporated herein by reference for a more detailed description of locus-specific primers.

Separation and Detection of DNA Fragments

Following amplification, products are then separated by electrophoresis, e.g., denaturing polyacrylamide gel electrophoresis (Sambrook et al., 1989). Preferred gel preparation and electrophoresis procedures are conducted as described in Example 1. Fragment separation occurs based on size and charge of the sample.

The DNA is then detected by, e.g., silver staining (Bassam et al. 1991). Alternatively, if radioactively-labeled or fluorescently-labeled primers were used for each locus, the products are detected by means available to detect these reporters as known to those skilled in the art. Amplified materials may be detected using any of a number of reporters including, e.g., silver staining, radioisotopes, fluorescers, chemiluminescers and enzymes in combination with detectable substrates.

Individual DNA samples containing amplified alleles are preferably compared with a size standard such as a DNA marker or locus-specific allelic ladder to determine the alleles present at each locus within the sample. The preferred size marker for evaluation of a multiplex amplification containing two or more polymorphic STR loci consists of a combination of allelic ladders for the loci being evaluated.

The preferred size marker for evaluation of a multiplex amplification containing two or more polymorphic STR loci which are generated using fluorescently-labeled primers for each locus consists of a combination of fluorescently-labeled allelic ladders for the loci being evaluated.

Following the construction of allelic ladders for individual loci, they may be mixed and loaded for gel electrophoresis at the same time as the loading of amplified samples occurs. Each allelic ladder co-migrates with alleles in the sample from the corresponding locus.

A permanent record of the data can be generated with the use of electrophoresis duplicating film (STR systems manual #TMD004, Promega Corporation, Madison, Wis.).

Advantage of Fluorescent Detection

With the advent of automated fluorescent imaging, faster detection and analysis of multiplex amplification products can be achieved. For fluorescent analyses, one fluoresceinated primer can be included in the amplification of each locus. Separation of the amplified fragments is achieved in precisely the same manner as with the silver stain detection method. The resulting gel is loaded onto a FluorImager® 575 (Molecular Dynamics, Sunnyvale, Calif.) which scans the gel and digitizes the data in three minutes. The FluorImager® contains an argon laser emitting 488 nm light which sweeps through the gel using a galvanometer-controlled mirror. The light activates fluorescent molecules in its path and they, in turn, emit light of higher wavelength. A filter prohibits passage of the original light, but allows collection of the emitted light by a fiber optic collector. A second filter selected by the user may be inserted between the fiber optic collector and the photomultiplier, allowing detection of specific wavelength bands (or colors) with each scan.

The image has an overall cleaner appearance than that obtained with the silver stain for three reasons. First, only one of the two PCR product strands is labeled with primer, simplifying the two band per allele images of the silver stain. Second, in the silver stain reaction, the entire gel is exposed to silver and prone to silver deposition causing a significant general background. With the fluorescent reporter, only the primer is labeled and the unincorporated primers migrate out of the bottom of the gel prior to detection. Third, some artifact bands of the PCR reaction are plentiful, but contain very little primer.

Because this fluorescent method detects only products with one particular primer, some of these artifacts which appear in silver stain of multiplex amplifications are not detected. In fact, this characteristic has allowed development of the more complex quadriplex as shown in FIG. **2** in place of the triplex shown in FIG. **1**.

Kit

The present invention is also directed to kits that utilize the process described. A basic kit includes a container having a locus-specific primer pair (or alternately separate containers containing each primer of a primer pair) for each locus. The kit also includes instructions for use.

Other ingredients may include an allelic ladder directed to each of the specified loci, a sufficient quantity of enzyme for amplification, amplification buffer to facilitate the amplification, loading solution for preparation of the amplified material for gel electrophoresis, human genomic DNA as a control to test that the system is working well, a size marker to insure that materials migrate as anticipated in the

gel, and a protocol and manual to educate the user and to limit error in use. The amounts of the various reagents in the kits can be varied depending upon a number of factors, such as the optimum sensitivity of the process. The instructions for use are suitable to enable any analyst to carry out the desired test. It is within the scope of this invention to provide test kits for use in manual applications or test kits for use with automated detectors or analyzers.

EXAMPLES

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the disclosure or protection granted by the patent.

Genomic DNA isolation and quantitation were performed essentially as described by Puers et al., 1993. These methods are generally known to those skilled in the art and are preferred, but not required, for application of the invention.

Amplification products were separated by electrophoresis through a 0.4mm thick 4% denaturing polyacrylamide gel (19:1 ratio of acrylamide to bis-acrylamide) which contained 7 M urea (Sambrook et al., 1989) and was chemically cross-linked to one glass plate (Kobayashi, 1988). DNA samples were mixed with 3 μl loading solution (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured at 95° C. for 2 min., and chilled on ice prior to loading.

Electrophoresis was performed at 60 W in 0.5× TBE for 1–2 hrs. The DNA was detected by silver staining (Bassam et al., 1991). Permanent images were obtained by exposure to Electrophoresis Duplicating Films (EDF, Kodak, Cat. No. 809 6232). Alternatively, detection can be performed by fluorescent scanning (Schumm et al., 1994) or radioactive detection (Hammond et al., 1994).

Example 1

Silver Stain Detection of Multiplex Amplification of Loci HUMCSF1PO, HUMTPOX, and HUMTH01

In this example, a DNA template (three DNA samples) was amplified at the individual loci HUMCSF1PO, HUMTPOX, and HUMTH01 simultaneously in a single reaction vessel. The PCR amplifications were performed in 50 μl volumes using 25 ng template, 0.03 U Taq DNA Polymerase/μl, 1×STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM MgCl2 and 200 μM each of dATP, dCTP, dGTP and dTTP), and using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 1 (96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 64° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 64° C. for 1 min., 70° C. for 1.5 min.) was employed.

Six amplification primers were used in combination, including 0.2 μM each HUMCSF1PO primers 1 [SEQ. ID. 5] and 2 [SEQ. ID. 6], 0.2 μM each HUMTPOX primers 1 [SEQ. ID. 29] and 2 [SEQ. ID. 30], and 0.6 μM each HUMTH01 primers 1 [SEQ. ID. 27] and 2 [SEQ. ID. 28].

Amplified products were separated by denaturing acrylamide gel electrophoresis on a 40 cm gel for 60–90 min. at 60 W and products were visualized by silver stain analysis according the protocol of *Bassam* et al. (1991).

Reference is made to FIG. **1** which reveals the silver stain detection of the multiplex amplification. Lanes 2, 3, and 5 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO, HUMTPOX, and HUMTH01. Lanes 1, 4, and 7 contain allelic ladders for the three loci and lane 6 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 2

Fluorescent Detection of Multiplex Amplification of Loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMVWFA31

In this example, a DNA template was amplified at the individual loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMVWFA31 simultaneously in a single reaction vessel. The PCR amplifications were performed in 25 μl volumes using 25 ng template, 0.04 U Taq DNA Polymerase/μl, 1×STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100, 1.5 mM MgCl2 and 200 μM each of DATP, dCTP, dGTP and dTTP), and using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 1, as described in Example 1, was employed. Eight amplification primers were used in combination, including 1 μM each HUMCSF1PO primer 2 [SEQ. ID 6] and fluorescein-labeled primer 1 [SEQ. ID. 5], 0.15 μM each HUMTPOX primer 1 [SEQ. ID. 29] and fluorescein-labeled primer 2 [SEQ. ID. 30], 0.2 μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27], and 1 μM each HUMVWFA31 primer 1 [SEQ. ID. 31] and fluorescein-labeled primer 2 [SEQ. ID. 32].

Amplified products were separated by denaturing acrylamide gel electrophoresis on a 32 cm gel for 45 minutes at 40 watts. Detection of the fluorescent signal was achieved using the FluorImager™ 575 (Molecular Dynamics, Sunnyvale, Calif.). Reference is made to FIG. **2** which is a computer image of a FluorImager scan. Lanes 2–7 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMVWFA31. Lane 1 contains allelic ladders for the 4 loci.

Example 3

Multiplex Amplification of Loci HUMHPRTB, HUMFESFPS, and HUMVWFA31

In this example, a DNA template was amplified at the loci HUMHPRTB, HUMFESFPS, and HUMVWFA31 simultaneously in a single reaction vessel. The PCR amplifications were performed in 25 μl volumes using 25 ng template, 0.03 U Taq DNA Polymerase/μl, 1×STR Buffer (described in example 1), and a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 2 (96° C. for 2 min., then 10 cycles of 94° C. for 1 min., 60° C. for 1 min., and 70° C. for 1.5 min., followed by 20 cycles of 90° C. for 1 min., 64° C. for 1 min., 70° C. for 1.5 min.) was employed. Amplified products were separated by denaturing acrylamide gel electrophoresis on a 32 cm gel for 45 min. at 40 W and products were visualized by silver stain analysis according the protocol of Bassam et al. (supra.). Six primers were used in combination including 0.2 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20], 1.5 μM each HUMFESFPS primers 1 [SEQ. ID. 17] and 2 [SEQ. ID. 18], and 1 μM each HUMVWFA31 primers 1 [SEQ. ID. 31] and 2 [SEQ. ID. 32].

Reference is made to FIG. **3** which reveals the silver stain detection of the multiplex amplification. Lanes 2–6 contain DNA samples simultaneously co-amplified for the loci

HUMHPRTB, HUMFESFPS, and HUMVWFA31. Lanes 1 and 7 contain allelic ladders for the 3 loci.

Example 4

Fluorescent Detection of Multiplex Amplification of Loci HUMHPRTB, HUMFESFPS, HUMBFXIII (F13B), and HUMLIPOL

In this example, a DNA template was amplified at the loci HUMHPRTB, HUMFESFPS, HUMBFXIII (F13B), and HUMLIPOL simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 2 using amplification protocol 2, as described in Example 3.

Eight primers were used in combination, including 1 μM each HUMHPRTB primer 2 [SEQ. ID. 20] and fluorescein-labeled primer 1 [SEQ. ID.19], 2.5 μM each HUMFESFPS primer 2 [SEQ. ID. 18] and fluorescein-labeled primer 1 [SEQ. ID. 17], 1 μM each HUMBFXIII (F13B) primer 2 [SEQ. ID. 14] and fluorescein-labeled primer 1 [SEQ. ID. 13], and 0.5 μM each HUMLIPOL primer 2 [SEQ. ID. 24] and fluorescein-labeled primer 1 [SEQ. ID. 23].

Reference is made to FIG. **4** which is a computer image of a FluorImager scan. Lanes 2–7 contain DNA samples simultaneously co-amplified for the loci HUMHPRTB, HUMFESFPS, HUMBFXIII (F13B), and HUMLIPOL. Lane 1 contains allelic ladders for the 4 loci.

Example 5

Multiplex Amplification of Loci HSAC04 (ACTBP2) and HUMCYP19

In this example, a DNA template was amplified at the individual loci HSAC04 and HUMCYP19 simultaneously in a single reaction vessel. The PCR amplifications were performed in 15 μl volumes with 25 ng template, 0.01 U Taq DNA Polymerase/μl, 1×Taq DNA Polymerase Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100 and 1.5 mM MgCl2) and 200 μM each of dATP, dCTP, dGTP and dTTP using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 2, as described in Example 3, was employed. Amplified products were separated and detected per example 1. Four primers were used in combination, including 1 μM each HSAC04 (ACTBP2) primers 1 [SEQ. ID. 1] and 2 [SEQ. ID. 2], and 1 μM each HUMCYP19 primers 1 [SEQ. ID. 7] and 2 [SEQ. ID. 8].

Reference is made to FIG. **5** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HSAC04 (ACTBP2) and HUMCYP19. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 6

Multiplex Amplification of Loci HSAC04 (ACTBP2), HUMCYP19, and HUMPLA2A1

In this example, a DNA template was amplified at the loci HSAC04 (ACTBP2), HUMCYP19, and HUMPLA2A1 simultaneously in a single reaction vessel. The PCR amplifications were performed in 15 μl volumes with 25 ng template, 0.02 U Taq DNA Polymerase/μl, 1×Taq DNA Polymerase Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton X-100 and 1.5 mM MgCl2) and 200 μM each of DATP, dCTP, dGTP and dTTP using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 2, as described in Example 3, was employed. Amplified products were separated and detected per example 1. Six primers were used in combination, including 1 μM each HSAC04 (ACTBP2) primers 1 [SEQ. ID. 1] and 2 [SEQ. ID. 2], 1 μM each HUMPLA2A1 primers 1 [SEQ. ID. 25] and 2 [SEQ. ID. 26], and 1 μM each HUMCYP19 primers 1 [SEQ. ID. 7] and 2 [SEQ. ID. 8].

Reference is made to FIG. **6** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HSAC04 (ACTBP2), HUMCYP19, and HUMPLA2A1. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 7

Multiplex Amplification of Loci HSAC04 (ACTBP2) and HUMFABP

In this example, a DNA template was amplified at the loci HSAC04 (ACTBP2) and HUMFABP simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 5 using amplification protocol 2, as described in Example 3. Four primers were used in combination, 1 μM each HSAC04 (ACTBP2) primers 1 [SEQ. ID. 1] and 2 [SEQ. ID. 2], and 1 μM each HUMFABP primers 1 [SEQ. ID. 15] and 2 [SEQ. ID. 16].

Reference is made to FIG. **7** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HSAC04 (ACTBP2) and HUMFABP. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 8

Multiplex Amplification of Loci HUMAPOA2, HUMCYP19, and HUMPLA2A1

In this example, a DNA template was amplified at the loci HUMAPOA2, HUMCYP19, and HUMPLA2A simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 6 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 1 μM each HUMAPOA2 primers 1 [SEQ. ID. 3] and 2 [SEQ. ID. 4], 1 μM each HUMCYP19 primers 1 [SEQ. ID. 7] and 2 [SEQ. ID. 8], and 1 μM each HUMPLA2A1 primers 1 [SEQ. ID. 25] and 2 [SEQ. ID. 26]. Reference is made to FIG. **8** which reveals the silver stain detection of the multiplex amplification. Lanes 1 and 3 contain DNA samples simultaneously co-amplified for the loci HUMAPOA2, HUMCYP19, and HUMPLA2A1. Lane 2 contains a DNA sample which failed to amplify and lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 9

Multiplex Amplification of Loci HUMCD4, HUMCSF1PO, and HUMTH01

In this example, a DNA template was amplified at the loci HUMCD4, HUMCSF1PO, and HUMTH01 simultaneously in a single reaction vessel. The PCR amplifications were performed in 50 μl volumes with 25 ng template, 0.02 U Taq DNA Polymerase/μl, 1×Taq DNA Polymerase Buffer (50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25° C.), 0.1% Triton

X-100 and 1.5 mM MgCl2) and 200 μM each of DATP, dCTP, dGTP and dTTP using a Thermal Cycler 480 (Perkin Elmer Cetus). Amplification protocol 1, as described in Example 1, was employed. Amplified products were separated and detected as described in Example 1. Six primers were used in combination, including 1 μM each HUMCD4 primers 1 [SEQ. ID. 9] and 2 [SEQ. ID. 10], 1 μM each HUMCSF1PO primers 1 [SEQ. ID. 5] and 2 [SEQ. ID. 6], and 1 μM each HUMTH01 primers 1 [SEQ. ID. 27] and 2 [SEQ. ID. 28].

Reference is made to FIG. **9** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMCD4, HUMCSF1PO, and HUMTH01. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 10

Multiplex Amplification of Loci HUMCYP19, HUMFABP, and HUMPLA2A1

In this example, a DNA template was amplified at the loci HUMCYP19, HUMFABP, and HUMPLA2A1 simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 6 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 1 μM each HUMCYP19 primers 1 [SEQ. ID. 7] and 2 [SEQ. ID. 8], 1 μM each HUMFABP primers 1 [SEQ. ID. 15] and 2 [SEQ. ID. 16] and 1 μM each HUMPLA2A1 primers 1 [SEQ. ID. 25] and 2 [SEQ. ID. 26].

Reference is made to FIG. **10** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMCYP19, HUMFABP, and HUMPLA2A1. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 11

Multiplex Amplification of Loci HUMCYP19, HUMHPRTB, and HUMPLA2A1

In this example, a DNA template was amplified at the loci HUMCYP19, HUMHPRTB, and HUMPLA2A1 simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 9 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 1 μM each HUMCYP19 primers 1 [SEQ. ID. 7] and 2 [SEQ. ID. 8], 1 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20], and 1 μM each HUMPLA2A1 primers 1 [SEQ. ID. 25] and 2 [SEQ. ID. 26].

Reference is made to FIG. **11** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMCYP19, HUMHPRTB, and HUMPLA2A1. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 12

Multiplex Amplification of Loci HUMF13A01 and HUMFABP

In this example, a DNA template was amplified at the loci HUMF13A01 and HUMFABP simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 5 using amplification protocol 1, as described in Example 1. Four primers were used in combination, including 1 μM each HUMF13A01 primers 1 [SEQ. ID. 11] and 2 [SEQ. ID. 12], and 1 μM each HUMFABP primers 1 [SEQ. ID. 15] and 2 [SEQ. ID. 16].

Reference is made to FIG. **12** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMF13A01 and HUMFABP. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 13

Multiplex Amplification of Loci HUMBFXIII (F13B) and HUMFESFPS

In this example, a DNA template was amplified at the loci HUMBFXIII (F13B) and HUMFESFPS simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 6 using amplification protocol 1, as described in Example 1. Four primers were used in combination, including 1 μM each HUMBFXIII (F13B) primers 1 [SEQ. ID. 13] and 2 [SEQ. ID. 14], and 1 μM each HUMFESFPS primers 1 [SEQ. ID. 17] and 2 [SEQ. ID. 18].

Reference is made to FIG. **13** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMBFXIII (F13B) and HUMFESFPS. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 14

Multiplex Amplification of Loci HUMBFXIII (F13B), HUMHPRTB, and HUMPLA2A1

In this example, a DNA template was amplified at the loci HUMBFXIII (F13B), HUMHPRTB, and HUMPLA2A1 simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 6 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 1 μM each HUMBFXIII (F13B) primers 1 [SEQ. ID. 13] and 2 [SEQ. ID. 14], 1 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20], and 1 μM each HUMPLA2A1 primers 1 [SEQ. ID. 25] and 2 [SEQ. ID. 26].

Reference is made to FIG. **14** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMBFXIII (F13B), HUMHPRTB, and HUMPLA2A1. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 15

Multiplex Amplification of Loci HUMF13A01, HUMFABP, and HUMCD4

In this example, a DNA template was amplified at the loci HUMF13A01, HUMFABP, and HUMCD4 simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 5 using amplification protocol 1, as described in Example 1. Six primers were used in combination, including 1 μM each HUMF13A01 primers 1 [SEQ. ID. 11] and 2 [SEQ. ID. 12],

1 μM each HUMFABP primers 1 [SEQ. ID. 15] and 2 [SEQ. ID. 16], and 1 μM each HUMCD4 primers 1 [SEQ. ID. 9] and 2 [SEQ. ID. 10].

Reference is made to FIG. **15** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMF13A01, HUMFABP, and HUMCD4. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 16

Multiplex Amplification of Loci HUMHPRTB and HUMFESFPS

In this example, a DNA template was amplified at the loci HUMHPRTB and HUMFESFPS simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using 500–0.5 ng template, 0.02 U Taq DNA Polymerase/μl and amplification protocol 2, as described in Example 3. Four primers were used in combination, including 0.2 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20] and 1.5 μM each HUMFESFPS primers 1 [SEQ. ID. 17] and 2 [SEQ. ID. 18].

Reference is made to FIG. **16** which reveals the silver stain detection of the multiplex amplification. Lanes 1–6 contain DNA samples simultaneously co-amplified for the loci HUMHPRTB and HUMFESFPS using 500, 50, 25, 5, 1 and 0.5 ng DNA template. Lane 7 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 17

Multiplex Amplification of Loci HUMHPRTB, HUMFESFPS, and HUMLIPOL

In this example, a DNA template was amplified at the loci HUMHPRTB, HUMFESFPS, and HUMLIPOL simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 0.4 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20], 3 μM each HUMFESFPS primers 1 [SEQ. ID. 17] and 2 [SEQ. ID. 18], and 2 μM each HUMLIPOL primers 1 [SEQ. ID. 23] and 2 [SEQ. ID. 24].

Reference is made to FIG. **17** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMHPRTB, HUMFESFPS and HUMLIPOL. Lane 4 displays a sample without DNA subjected to the same procedures, i.e., a negative control.

Example 18

Multiplex Amplification of Loci HUMBFXIII (F13B) and HUMLIPOL

In this example, a DNA template was amplified at the loci HUMBFXIII (F13B) and HUMLIPOL Simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using 0.02 U Taq DNA Polymerase/μl and amplification protocol 2, as described in Example 3. Four primers were used in combination, including 1 μM each HUMBFXIII (F13B) primers 1 [SEQ. ID. 13] and 2 [SEQ. ID. 14] and 1 μM each HUMLIPOL primers 1 [SEQ. ID. 23] and 2 [SEQ. ID. 24].

Reference is made to FIG. **18** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMBFXIII (F13B) and HUMLIPOL.

Example 19

Multiplex Amplification of Loci HUMHPRTB, HUMTPOX, and HUMBFXIII (F13B)

In this example, a DNA template was amplified at the loci HUMHPRTB, HUMTPOX, and HUMBFXIII (F13B) simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 1 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20], 0.2 μM each HUMTPOX primers 1 [SEQ. ID. 29] and 2 [SEQ. ID. 30], and 2 μM each HUMBFXIII (F13B) primers 1 [SEQ. ID. 13] and 2 [SEQ. ID. 14].

Reference is made to FIG. **19** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMHPRTB, HUMTPOX, and HUMBFXIII (F13B).

Example 20

Multiplex Amplification of Loci HUMHPRTB, HUMFESFPS, and HUMBFXIII (F13B)

In this example, a DNA template was amplified at the loci HUMHPRTB, HUMFESFPS, and HUMBFXIII (F13B) simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 1 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20], 2 μM each HUMFESFPS primers 1 [SEQ. ID. 17] and 2 [SEQ. ID. 18], and 2 μM each HUMBFXIII (F13B) primers 1 [SEQ. ID. 13] and 2 [SEQ. ID. 14].

Reference is made to FIG. **20** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMHPRTB, HUMFESFPS, and HUMBFXIII (F13B).

Example 21

Multiplex Amplification of Loci HUMCSF1PO, HUMTPOX, and HUMCD4

In this example, a DNA template was amplified at the loci HUMCSF1PO, HUMTPOX, and HUMCD4 simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using amplification protocol 1, as described in Example 1. Six primers were used in combination, including 1 μM each HUMCSF1PO primers 1 [SEQ. ID. 5] and 2 [SEQ. ID. 6], 1 μM each HUMTPOX primers 1 [SEQ. ID. 29] and 2 [SEQ. ID. 30], and 1 μM each HUMCD4 primers 1 [SEQ. ID. 9] and 2 [SEQ. ID. 10].

Reference is made to FIG. **21** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO, HUMTPOX, and HUMCD4.

Example 22

Multiplex Amplification of Loci HUMHPRTB, HUMFESFPS, and HUMMYOPK (Myotonic)

In this example, a DNA template was amplified at the loci HUMHPRTB, HUMFESFPS, and HUMMYOPK simulta-

neously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using amplification protocol 2, as described in Example 3. Six primers were used in combination, including 1 μM each HUMHPRTB primers 1 [SEQ. ID. 19] and 2 [SEQ. ID. 20], 1 μM each HUMFESFPS primers 1 [SEQ. ID. 17] and 2 [SEQ. ID. 18], and 1 μM each HUMMYOPK (Myotonic) primers 1 [SEQ. ID. 21] and 2 [SEQ. ID. 22].

Reference is made to FIG. **22** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMHPRTB, HUMFESFPS, and HUMMYOPK (Myotonic).

Example 23

Multiplex Amplification of Loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4

In this example, a DNA template was amplified at the loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4 simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using 0.04 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Eight primers were used in combination, including 1 μM each HUMCSF1PO primers 1 [SEQ. ID. 5] and 2 [SEQ. ID. 6], 1 μM each HUMTPOX primers 1 [SEQ. ID. 29] and 2 [SEQ. ID. 30], 1 μM each HUMTH01 primers 1 [SEQ. ID. 27] and 2 [SEQ. ID. 28], and 1 μM each HUMCD4 primers 1 [SEQ. ID. 9] and 2 [SEQ. ID. 10].

Reference is made to FIG. **23** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4.

Example 24

Multiplex Amplification of Loci HUMF13A01 and HUMMYOPK (Myotonic)

In this example, a DNA template was amplified at the loci HUMF13A01 and HUMMYOPK (Myotonic) simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using 0.04 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Four primers were used in combination, including 0.1 μM each HUMF13A01 primers 1 [SEQ. ID. 11] and 2 [SEQ. ID. 12] and 1 μM each HUMMYOPK (Myotonic) primers 1 [SEQ. ID. 21] and 2 [SEQ. ID. 22].

Reference is made to FIG. **24** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMF13A01 and HUMMYOPK (Myotonic).

Example 25

Multiplex Amplification of Loci HUMF13A01 and HUMBFXIII (F13B)

In this example, a DNA template was amplified at the loci HUMF13A01 and HUMBFXIII (F13B) simultaneously in a single reaction vessel. The PCR amplifications and other manipulations were performed as described in Example 1 using 0.03 U Taq DNA Polymerase/μl and amplification protocol 2, as described in Example 3. Four primers were used in combination, including 0.1 μM each HUMF13A01 primers 1 [SEQ. ID. 11] and 2 [SEQ. ID. 12] and 0.5 μM each HUMBFXIII (F13B) primers 1 [SEQ. ID. 13] and 2 [SEQ. ID. 14].

Reference is made to FIG. **25** which reveals the silver stain detection of the multiplex amplification. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMF13A01 and HUMBFXIII (F13B).

Example 26

Fluorescent Detection of Multiplex Amplification of Loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4

In this example, a DNA template was amplified at the individual loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4 simultaneously in a single reaction vessel. The PCR amplifications were performed as described in Example 1 using 0.04 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Eight amplification primers were used in combination, including 2 μM each HUMCSF1PO primer 2 [SEQ. ID. 6] and fluorescein-labeled primer 1 [SEQ. ID. 5], 0.5 μM each HUMTPOX primer 1 [SEQ. ID. 29] and fluorescein-labeled primer 2 [SEQ. ID. 30], 0.5 μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27] and 0.5 μM each HUMCD4 primer 1 [SEQ. ID. 9] and fluorescein-labeled primer 2 [SEQ. ID. 10].

Amplified products were detected as in Example 2. Reference is made to FIG. **26** which is a photograph of a computer image of a FluorImager scan. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO, HUMTPOX, HUMTH01, and HUMCD4.

Example 27

Fluorescent Detection of Multiplex Amplification of Loci HUMCSF1PO, HUMTH01, and HUMCD4

In this example, a DNA template was amplified at the individual loci HUMCSF1PO, HUMTH01, and HUMCD4 simultaneously in a single reaction vessel. The PCR amplifications were performed as described in Example 1 using 0.02 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Six amplification primers were used in combination, including 1 μM each HUMCSF1PO primer 2 [SEQ. ID. 6] and fluorescein-labeled primer 1 [SEQ. ID. 5], 1 μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27] and 1 μM each HUMCD4 primer 1 [SEQ. ID. 9] and fluorescein-labeled primer 2 [SEQ. ID. 10].

Amplified products were detected as in Example 2. Reference is made to FIG. **27** which is a photograph of a computer image of a FluorImager scan. Lanes 1 and 2 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO, HUMTH01, and HUMCD4.

Example 28

Fluorescent Detection of Multiplex Amplification of Loci HUMCSF1PO, HUMTH01, and HUMVWFA31

In this example, a DNA template was amplified at the individual loci HUMCSF1PO, HUMTH01, and HUMVWFA31 simultaneously in a single reaction vessel. The PCR amplifications were performed as described in Example 1 using 0.02 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Six

amplification primers were used in combination, including 1 μM each HUMCSF1PO primer 2 [SEQ. ID. 6] and fluorescein-labeled primer 1 [SEQ. ID. 5], 1 μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27], and 1 μM each HUMVWFA31 primer 1 [SEQ. ID. 31] and fluorescein-labeled primer 2 [SEQ. ID. 32].

Amplified products were detected as in Example 2. Reference is made to FIG. **28** which is a photograph of a computer image of a FluorImager scan. Lanes 1 and 2 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO, HUMTH01, and HUMVWFA31.

Example 29

Fluorescent Detection of Multiplex Amplification of Loci HUMHPRTB, HUMBFXIII (F13B), and HUMLIPOL

In this example, a DNA template was amplified at the individual loci HUMHPRTB, HUMBFXIII (F13B), and HUMLIPOL simultaneously in a single reaction vessel. The PCR amplifications were performed as described in Example 1 using 0.03 U Taq DNA Polymerase/μl and amplification protocol 2, as described in Example 3. Six amplification primers were used in combination, including 1 μM each HUMHPRTB primer 2 [SEQ. ID. 20] and fluorescein-labeled primer 1 [SEQ. ID. 19], 1 μM each HUMBFXIII (F13B) primer 2 [SEQ. ID. 14] and fluorescein-labeled primer 1 [SEQ. ID. 13], and 1 μM each HUMLIPOL primer 2 [SEQ. ID. 24] and fluorescein-labeled primer 1 [SEQ. ID. 23].

Amplified products were detected as in Example 2. Reference is made to FIG. **29** which is a photograph of a computer image of a FluorImager scan. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMHPRTB, HUMBFXIII (F13B), and HUMLIPOL.

Example 30

Fluorescent Detection of Multiplex Amplification of Loci HUMCSF1PO and HUMTH01

In this example, a DNA template was amplified at the individual loci HUMCSF1PO and HUMTH01 simultaneously in a single reaction vessel. The PCR amplifications were performed as described in Example 1 using 0.02 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Four amplification primers were used in combination, including 2 μM each HUMCSF1PO primer 2 [SEQ. ID. 6] and fluorescein-labeled primer 1 [SEQ. ID. 5] and 1 μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27].

Amplified products were detected as in Example 2. Reference is made to FIG. **30** which is a photograph of a computer image of a FluorImager scan. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMCSF1PO and HUMTH01.

Example 31

Fluorescent Detection of Multiplex Amplification of Loci HUMTH01 and HUMCD4

In this example, a DNA template was amplified at the individual loci HUMTH01 and HUMCD4 simultaneously in a single reaction vessel. The PCR amplifications were performed as described in Example 1 using 0.02 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Four amplification primers were used in combination, including 1 μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27] and 1 μM each HUMCD4 primer 1 [SEQ. ID. 9] and fluorescein-labeled primer 2 [SEQ. ID. 10].

Amplified products were detected as in Example 2. Reference is made to FIG. **31** which is a photograph of a computer image of a FluorImager scan. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMTH01 and HUMCD4.

Example 32

Fluorescent Detection of Multiplex Amplification of Loci HUMTH01 and HUMTPOX

In this example, a DNA template was amplified at the individual loci HUMTH01 and HUMTPOX simultaneously in a single reaction vessel. The PCR amplifications were performed as described in Example 1 using 0.02 U Taq DNA Polymerase/μl and amplification protocol 1, as described in Example 1. Four amplification primers were used in combination, including 1 μM each HUMTH01 primer 2 [SEQ. ID. 28] and fluorescein-labeled primer 1 [SEQ. ID. 27] and 1 μM each HUMTPOX primer 2 [SEQ. ID. 30] and fluorescein-labeled primer 1 [SEQ. ID. 29].

Amplified products were detected as in Example 2. Reference is made to FIG. **32** which is a photograph of a computer image of a FluorImager scan. Lanes 1–3 contain DNA samples simultaneously co-amplified for the loci HUMTH01 and HUMTPOX.

It is understood that the invention is not confined to the particular construction and arrangements herein illustrated and described, but embraces such modified forms thereof and come within the scope of the claims following the bibliography.

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trophy families, using dinucleotide repeat polymorphisms," *Am J. Hum. Genet*. 49: 951–960.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 32

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACATCTCCCC TACCGCTATA 20

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AATCTGGGCG ACAAGAGTGA 20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGAGCAGTCC TAGGGCCGCG CCGT 24

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTGACAGAGG GAGACTCCAT TAAA 24

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AACCTGAGTC TGCCAAGGAC TAGC 24

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TTCCACACAC CACTGGCCAT CTTC 24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GCAGGTACTT AGTTAGCTAC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TTACAGTGAG CCAAGGTCGT 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCAGGAAGTT GAGGCTGCAG TGAA 24

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTGGAGTCGC AAGCTGAACT AGCG 24

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GAGGTTGCAC TCCAGCCTTT GCAA 24

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TTCCTGAATC ATCCCAGAGC CACA 24

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TGAGGTGGTG TACTACCATA 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GATCATGCCA TTGCACTCTA 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GTAGTATCAG TTTCATAGGG TCACC 25

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CAGTTCGTTT CCATTGTCTG TCCG 24

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

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GCTGTTAATT CATGTAGGGA AGGC 24

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GTAGTCCCAG CTACTTGGCT ACTC 24

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATGCCACAGA TAATACACAT CCCC 24

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CTCTCCAGAA TAGTTAGATG TAGG 24

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GCTCGAAGGG TCCTTGTAGC CGGG 24

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GATAGGTGGG GGTGCGTGGA GGAT 24

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(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CTGACCAAGG ATAGTGGGAT ATAG 24

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGTAACTGAG CGAGACTGTG TCT 23

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGTTGTAAGC TCCATGAGGT TAGA 24

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TTGAGCACTT ACTATGTGCC AGGCT 25

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GTGGGCTGAA AAGCTCCCGA TTAT 24

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

ATTCAAAGGG TATCTGGGCT CTGG 24

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ACTGGCACAG AACAGGCACT TAGG 24

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GGAGGAACTG GGAACCACAC AGGT 24

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GAAAGCCCTA GTGGATGATA AGAATAATC 29

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

GGACAGATGA TAAATACATA GGATGGATGG 30

What is claimed is:

1. A method of simultaneously determining the alleles present in at least three short tandem repeat loci from one or more DNA samples, comprising:

a) obtaining at least one DNA sample to be analyzed;

b) selecting a set of at least three short tandem repeat loci of the DNA sample to be analyzed which can be co-amplified, wherein the at least three short tandem repeat loci in the set comprises at least three loci selected from the group consisting of:

HUMPOX, HUMTH01 and HUMCD4;

HUMTPOX, HUMTH01 and HUMVWFA31;

HUMHPRTB, HUMFESFPS and HUMVWFA31;

HSAC04 (ACTBP2), HUMCYP19 and HUMPLA2A1;
HUMAPOA2, HUMCYP19 and HUMPLA2A1;
HUMCD4, HUMCSF1PO and HUMTH01;
HUMCYP19, HUMFABP and HUMPLA2A1;
HUMCYP19, HUMHPRTB and HUMPLA2A1;
HUMHPRTB, HUMFESFPS and HUMLIPOL;
HUMF13AO1, HUMFABP and HUMCD4;
HUMHPRTB, HUMBFXIII (F13B) and HUMPLA2A1;
HUMHPRTB, HUMBFXIII (F13B) and HUMTPOX;
HUMHPRTB, HUMBFXIII (F13B) and HUMFESFPS;
HUMCSF1PO, HUMTPOX and HUMCD4;
HUMHPRTB, HUMFESFPS and HUMMYOPK (Myotonic);
HUMCSF1PO, HUMTH01 and HUMCD4;
HUMCSF1PO, HUMTH01 and HUMVWFA31; and
HUMHPRTB, HUMBFXIII (F13B) and HUMLIPOL;

c) co-amplifying the set of at least three short tandem repeat loci in a multiplex amplification reaction, thereby producing a mixture of amplified alleles from each of the co-amplified loci in the set; and

d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

2. The method of claim **1** wherein in step (b), the at least three loci are co-amplified by multiplex polymerase chain reaction.

3. The method of claim **1** wherein the at least three loci are co-amplified using at least one oligonucleotide primer pair consisting of two oligonucleotide primers, at least one of which has a sequence selected from a group of sequences consisting of:

SEQ ID. NO. 1 and SEQ ID. NO. 2 when one of the loci in the set is HSAC04;
SEQ ID. NO. 3 and SEQ ID. NO. 4 when one of the loci in the set is HUMAPOA2;
SEQ ID. NO. 5 and SEQ ID. NO. 6 when one of the loci in the set is HUMCSF1PO;
SEQ ID. NO. 7 and SEQ ID. NO. 8 when one of the loci in the set is HUMCYP19;
SEQ ID. NO. 9 and SEQ ID. NO. 10 when one of the loci in the set is HUMCD4;
SEQ ID. NO. 11 and SEQ ID. NO. 12 when one of the loci in the set is HUMF13A01;
SEQ ID. NO.13 and SEQ ID. NO. 14 when one of the loci in the set is HUMBFXIII;
SEQ ID. NO. 15 and SEQ ID. NO. 16 when one of the loci in the set is HUMFABP;
SEQ ID. NO. 17 and SEQ ID. NO. 18 when one of the loci in the set is HUMFESFPS;
SEQ ID. NO. 19 and SEQ ID. NO. 20 when one of the loci in the set is HUMHPRTB;
SEQ ID. NO. 21 and SEQ ID. NO. 22 when one of the loci in the set is HUMMYOPK (Myotonic);
SEQ ID. NO. 23 and SEQ ID. NO. 24 when one of the loci in the set is HUMLIPOL;
SEQ ID. NO. 25 and SEQ ID. NO. 26 when one of the loci in the set is HUMPLA2A1;
SEQ ID. NO. 27 and SEQ ID. NO. 28 when one of the loci in the set is HUMTH01;
SEQ ID. NO. 29 and SEQ ID. NO. 30 when one of the loci in the set is HUMTPOX; and
SEQ ID. NO. 31 and SEQ ID. NO. 32 when one of the loci in the set is HUMVWFA31.

4. The method of claim **1**, wherein the amplified alleles are evaluated in step (d) by separating the alleles and comparing the separated alleles to a size standard selected from a DNA size marker or a locus-specific allelic ladder.

5. The method of claim **1**, further comprising the step of separating the alleles by denaturing polyacrylamide gel electrophoresis.

6. The method of claim **5** wherein the separated alleles are detected by silver staining.

7. The method of claim **5** wherein the separated alleles are detected by fluorescence detection.

8. The method of claim **1**, further comprising:

identifying primers for co-amplifying each locus in the set of loci selected in step (b) such that the amplified alleles produced in the multiplex amplification reaction of step (c) do not overlap when separated to evaluate the amplified alleles in step (e); and

using the primers in the multiplex amplification reaction in step (c).

9. The method of claim **1** wherein the at least one DNA sample to be analyzed is selected from the group consisting of blood, semen, vaginal cells, hair, saliva, urine or other tissue, placental cells or fetal cells present in amniotic fluid and mixtures of body fluids.

10. A kit for simultaneously analyzing short tandem repeat sequences in at least three loci, comprising:

a single container containing oligonucleotide primers for each locus in a set of at least three short tandem repeat loci, wherein the at least three short tandem repeat loci in the set comprises at least three loci selected from the group consisting of:
HUMTPOX, HUMTH01 and HUMCD4;
HUMTPOX, HUMTH01 and HUMVWFA31;
HUMTPOX, HUMVWFA31 and HUMCSF1PO;
HUMHPRTB, HUMFESFPS and HUMVWFA31;
HSAC04 (ACTBP2), HUMCYP19 and HUMPLA2A1;
HUMAPOA2, HUMCYP19 and HUMPLA2A1;
HUMCD4, HUMCSF1PO and HUMTH01;
HUMCYP19, HUMFABP and HUMPLA2A1;
HUMCYP19, HUMHPRTB and HUMPLA2A1;
HUMHPRTB, HUMFESFPS and HUMLIPOL;
HUMF13AO1, HUMFABP and HUMCD4;
HUMHPRTB, HUMBFXIII (F13B) and HUMPLA2A1;
HUMHPRTB, HUMBFXIII (F13B) and HUMTPOX;
HUMHPRTB, HUMBFXIII (F13B) and HUMFESFPS;
HUMBFXIII (F13B), HUMFESFPS and HUMLIPOL;
HUMCSF1PO, HUMTPOX and HUMCD4;
HUMBRTB, HUMFESFPS and HUMMYOPK (Myotonic);
HUMCSF1PO, HUMTH01 and HUMCD4;
HUMCSF1PO, HUMTH01 and HUMVWFA31; and
HUMHPRTB, HUMBFXIII (F13B) and HUMLIPOL.

11. The kit of claim **10** wherein each of the oligonucleotide primers in the kit is designed to hybridize with an allele of one of the loci in the set of at least two short tandem repeat loci, wherein the sequence of at least one of the primers is selected from the group consisting of:

SEQ ID. NO. 1 and SEQ ID. NO. 2 when one of the loci in the set is HSAC04;
SEQ ID. NO. 3 and SEQ ID. NO. 4 when one of the loci in the set is HUMAPOA2;

SEQ ID. NO. 5 and SEQ ID. NO. 6 when one of the loci in the set is HUMCSF1PO;

SEQ ID. NO. 7 and SEQ ID. NO. 8 when one of the loci in the set is HUMCYP19;

SEQ ID. NO. 9 and SEQ ID. NO. 10 when one of the loci in the set is HUMCD4;

SEQ ID. NO. 11 and SEQ ID. NO. 12 when one of the loci in the set is HUMF13A01;

SEQ ID. NO. 13 and SEQ ID. NO. 14 when one of the loci in the set is HUMBFXIII;

SEQ ID. NO. 15 and SEQ ID. NO. 16 when one of the loci in the set is HUMFABP;

SEQ ID. NO. 17 and SEQ ID. NO. 18 when one of the loci in the set is HUMFESFPS;

SEQ ID. NO. 19 and SEQ ID. NO. 20 when one of the loci in the set is HUMBPRTB;

SEQ ID. NO. 21 and SEQ ID. NO. 22 when one of the loci in the set is HUMMYOPK (Myotonic);

SEQ ID. NO. 23 and SEQ ID. NO. 24 when one of the loci in the set is HUMLIPOL;

SEQ ID. NO. 25 and SEQ ID. NO. 26 when one of the loci in the set is HUMPLA2A1;

SEQ ID. NO. 27 and SEQ ID. NO. 28 when one of the loci in the set is HUMTH01;

SEQ ID. NO. 29 and SEQ ID. NO. 30 when one of the loci in the set is HUMTPOX; and

SEQ ID. NO. 31 and SEQ ID. NO. 32 when one of the loci in the set is HUMVWFA31.

12. A method of simultaneously determining the alleles present in a set of short tandem repeat loci from one or more DNA samples, comprising:

a) obtaining at least one DNA sample to be analyzed;

b) selecting a set of short tandem repeat loci of the DNA sample to be analyzed which can be co-amplified, comprising HUMCSF1PO, HUMTPOX, and HUMTH01;

c) co-amplifying the set of short tandem repeat loci in a multiplex amplification reaction, thereby producing a mixture of amplified alleles from each of the co-amplified loci in the set; and

d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

13. The method of claim **12**, wherein the multiplex reaction is carried out using oligonucleotide primer pairs with primer pair sequences comprising: SEQ ID. NO. 5 and SEQ ID. NO. 6; SEQ ID. NO. 29 and SEQ ID. NO. 30; and SEQ ID. NO. 27 and SEQ ID. NO. 28.

14. The method of claim **12**, wherein the oligonucleotide primer pairs having the sequences SEQ ID. NO. 5 and SEQ ID. NO. 6, and SEQ ID. NO. 29 and SEQ ID. NO. 30 are present in a concentration of about 0.2 μM, and the oligonucleotide primer pairs SEQ ID. NO. 27 and SEQ ID. NO. 28 are present in a concentration of about 0.6 μM.

15. The method of claim **12**, wherein the set of loci co-amplified further comprises HUMVWFA31.

16. The method of claim **12**, wherein the multiplex reaction is carried out using oligonucleotide primer pairs with primer pair sequences comprising: SEQ ID. NO. 5 and SEQ ID. NO. 6, SEQ ID. NO. 29 and SEQ ID. NO. 30, SEQ ID. NO. 27 and SEQ ID. NO. 28, and SEQ ID. NO. 31 and SEQ ID. NO. 32.

17. The method of claim **16**, wherein the oligonucleotide primer pairs SEQ ID. NO. 5 and SEQ ID. NO. 6 are present in a concentration of about 1 μM; oligonucleotide primer pairs SEQ ID. NO. 29 and SEQ ID. NO. 30 are present in a concentration of about 0.15 μM, oligonucleotide primer pairs SEQ ID. NO. 27 and SEQ ID. NO. 28 are present in a concentration of about 0.2 μM, and oligonucleotide primer pair SEQ ID. NO. 31 and SEQ ID. NO. 32 are present in a concentration of about 1 μM.

18. The method of claim **12**, wherein the amplified alleles are separated by denaturing polyacrylamide gel electrophoresis, and detected by silver staining.

19. The method of claim **12**, wherein the multiplex amplification reaction includes oligonucleotide primers for each locus in the set of loci selected in step (b), wherein at least one of the oligonucleotide primers for each locus is fluorescently labeled.

20. The method of claim **12**, wherein the set of loci co-amplified further comprises HUMCD4.

21. The method of claim **12**, wherein the set of loci co-amplified further comprises HUMVWFA31.

22. The method of claim **12**, wherein the amplified alleles are separated by denaturing polyacrylamide gel electrophoresis, and detected by fluorescent detection.

23. A kit for simultaneously analyzing short tandem repeat sequences in a set of short tandem repeat loci from one or more DNA samples, comprising:

a single container containing oligonucleotide primers for each locus in a set of short tandem repeat loci which can be co-amplified, comprising HUMCSF1PO, HUMTPOX, and HUMTH01.

24. The kit of claim **23**, wherein the kit contains oligonucleotide primers designed to co-amplify the set of short tandem repeat loci, further comprising HUMVWFA31.

25. The kit of claim **23**, wherein the kit contains oligonucleotide primers designed to co-amplify the set of short tandem repeat loci, further comprising HUMCD4.

26. The kit of claim **23**, wherein each of the oligonucleotide primers in the kit is designed to hybridize with an allele of one of the loci in the set of at least three short tandem repeat loci, wherein at least one of the oligonucleotide primers in the kit has a sequence selected from the group consisting of: SEQ ID. NO. 5, SEQ ID. NO. 6, SEQ ID. NO. 29, SEQ ID. NO. 30, SEQ ID. NO. 27, and SEQ ID. NO. 28.

27. The kit of claim **23**, wherein one of each of the pair of oligonucleotide primers in the kit is fluorescently-labeled.

28. A method of simultaneously determining the alleles present in a set of short tandem repeat loci from one or more DNA samples, comprising:

a) obtaining at least one DNA sample to be analyzed;

b) selecting a set of short tandem repeat loci of the DNA sample to be analyzed which can be co-amplified, comprising HUMTPOX, HUMVWFA31, and HUMCSF1PO;

c) co-amplifying the set of short tandem repeat loci in a multiplex amplification reaction, thereby producing a mixture of amplified alleles from each of the co-amplified loci in the set; and

d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

29. The method of claim **28**, wherein the multiplex reaction is carried out using oligonucleotide primer pairs with at least one primer pair selected from the group of primer pair sequences consisting of: SEQ ID. NO. 29 and SEQ ID. NO. 30; SEQ ID. NO. 31 and SEQ ID. NO. 32; and SEQ ID. NO. 5 and SEQ ID. NO. 6.

30. The method of claim **28**, wherein the amplified alleles are separated by denaturing polyacrylamide gel electrophoresis, and detected by silver staining.

31. The method of claim **28**, wherein the amplified alleles are separated by denaturing polyacrylamide gel electrophoresis, and detected by fluorescent analysis.

32. The method of claim **31**, wherein the multiplex amplification reaction includes oligonucleotide primers for each locus in the set of loci selected in step (b), wherein at least one of the oligonucleotide primers for each locus is fluorescently labeled.

33. A kit for simultaneously analyzing short tandem repeat sequences in a set of short tandem repeat loci from one or more DNA samples, comprising:

a single container containing oligonucleotide primers for each locus in a set of short tandem repeat loci which can be co-amplified, comprising HUMTPOX, HUMVWFA31, and HUMCSF1PO.

34. The kit of claim **33**, wherein each of the oligonucleotide primers in the kit is designed to hybridize with an allele of one of the loci in the set of at least three short tandem repeat loci, wherein at least one of the oligonucleotide primers in the kit has a sequence selected from the group consisting of: SEQ ID. NO. 29 and SEQ ID. NO. 30; SEQ ID. NO. 31 and SEQ ID. NO. 32; and SEQ ID. NO. 5 and SEQ ID. NO. 6.

35. A method of simultaneously determining the alleles present in a set of short tandem repeat loci from one or more DNA samples, comprising:

a) obtaining at least one DNA sample to be analyzed;

b) selecting a set of short tandem repeat loci of the DNA sample to be analyzed which can be co-amplified, comprising HUMBFXIII (F13B), HUMFESFPS, and HUMLIPOL;

c) co-amplifying the set of short tandem repeat loci in a multiplex amplification reaction, thereby producing a mixture of amplified alleles from each of the co-amplified loci in the set; and

d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

36. The method of claim **35**, wherein the multiplex reaction is carried out using oligonucleotide primer pairs with at least one primer pair selected from the group of primer pair sequences consisting of: SEQ ID. NO. 13 and SEQ ID. NO. 14; SEQ ID. NO. 17 and SEQ ID. NO. 18; and SEQ ID. NO. 23 and SEQ ID. NO. 24.

37. The method of claim **35**, wherein the set of short tandem repeat loci selected for multiplex amplification further comprises HUMHPRTB.

38. The method of claim **35**, wherein the amplified alleles are separated by denaturing polyacrylamide gel electrophoresis, and detected by silver staining.

39. The method of claim **35**, wherein the amplified alleles are separated by denaturing polyacrylamide gel electrophoresis, and detected by fluorescent analysis.

40. The method of claim **35**, wherein the multiplex amplification reaction includes oligonucleotide primers for each locus in the set of loci selected in step (b), wherein at least one of the oligonucleotide primers for each locus is fluorescently labeled.

41. A kit for simultaneously analyzing short tandem repeat sequences in a set of short tandem repeat loci from one or more DNA samples, comprising:

a single container containing oligonucleotide primers for each locus in a set of short tandem repeat loci which can be co-amplified, comprising HUMBFXIII (F13B), HUMFESFPS, and HUMLIPOL.

42. The kit of claim **41**, wherein each of the oligonucleotide primers in the kit is designed to hybridize with an allele of one of the loci in the set of at least three short tandem repeat loci, wherein at least one of the oligonucleotide primers in the kit has a sequence selected from the group consisting of: SEQ ID. NO. 13 and SEQ ID. NO. 14; SEQ ID. NO. 17 and SEQ ID. NO. 18; and SEQ ID. NO. 23 and SEQ ID. NO. 24.

43. The kit of claim **41**, wherein the kit contains oligonucleotide primers designed to co-amplify the set of short tandem repeat loci, further comprising HUMHPRTB.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,221,598 B1
DATED : April 24. 2001
INVENTOR(S) : James W. Schumm et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 12,
Line 20, "DATP" should read -- dATP --.

Column 13,
Line 66, "DATP" should read -- dATP --.

Column 15,
Line 1, "DATP" should read -- dATP --.

Column 38,
Line 53, "HUMBRTB" should read -- HUMHPRTB --.

Column 39,
Line 17, "HUMBPRTB" should read -- HUMHPRTB --.

Signed and Sealed this

Eleventh Day of December, 2001

Attest:

Nicholas P. Godici

Attesting Officer

NICHOLAS P. GODICI
Acting Director of the United States Patent and Trademark Office

EXHIBIT 8


US006479235B1

(12) **United States Patent**
Schumm et al.

(10) **Patent No.: US 6,479,235 B1**
(45) **Date of Patent: Nov. 12, 2002**

(54) **MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI**

(75) Inventors: **James W. Schumm**; **Cynthia J. Sprecher**, both of Madison, WI (US)

(73) Assignee: **Promega Corporation**, Madison, WI (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/199,542**

(22) Filed: **Nov. 25, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/632,575, filed on Apr. 15, 1996, now Pat. No. 5,843,660, which is a continuation-in-part of application No. 08/316,544, filed on Sep. 30, 1994.

(51) **Int. Cl.**[7] **1C12Q 1/68**; C12P 19/34; C07H 21/04; C07H 21/02

(52) **U.S. Cl.** **435/6**; 435/91.2; 435/91.5; 536/23.1; 536/24.33

(58) **Field of Search** 435/91.2, 91.5, 435/91.1, 6, 5; 536/23.1, 24.3, 24.31, 24.33; 935/77, 78

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(57) **ABSTRACT**

Methods and materials are disclosed for use in simultaneously amplifying at least thirteen loci of genomic DNA in a single multiplex reaction, as are methods and materials for use in the analysis of the products of such reactions. Included in the present invention are materials and methods for the simultaneous amplification of at least thirteen short tandem repeat loci, including specific materials and methods for the analysis of thirteen such loci specifically selected by the United States Federal Bureau of Investigation as core loci for use in the Combined DNA Index System (CODIS) database.

24 Claims, 12 Drawing Sheets

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FIG. 1A

Amplification with DNA template

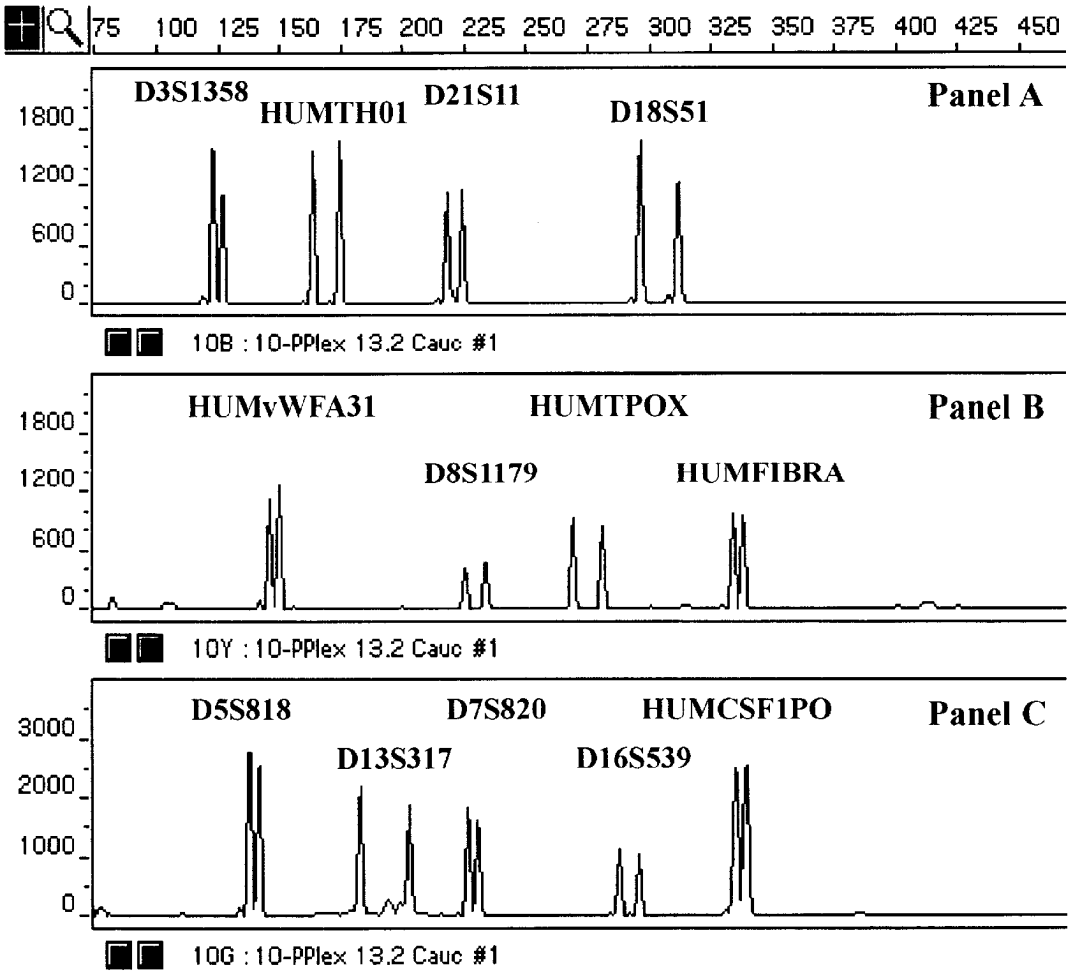


FIG. 1B

Amplification with NO DNA template

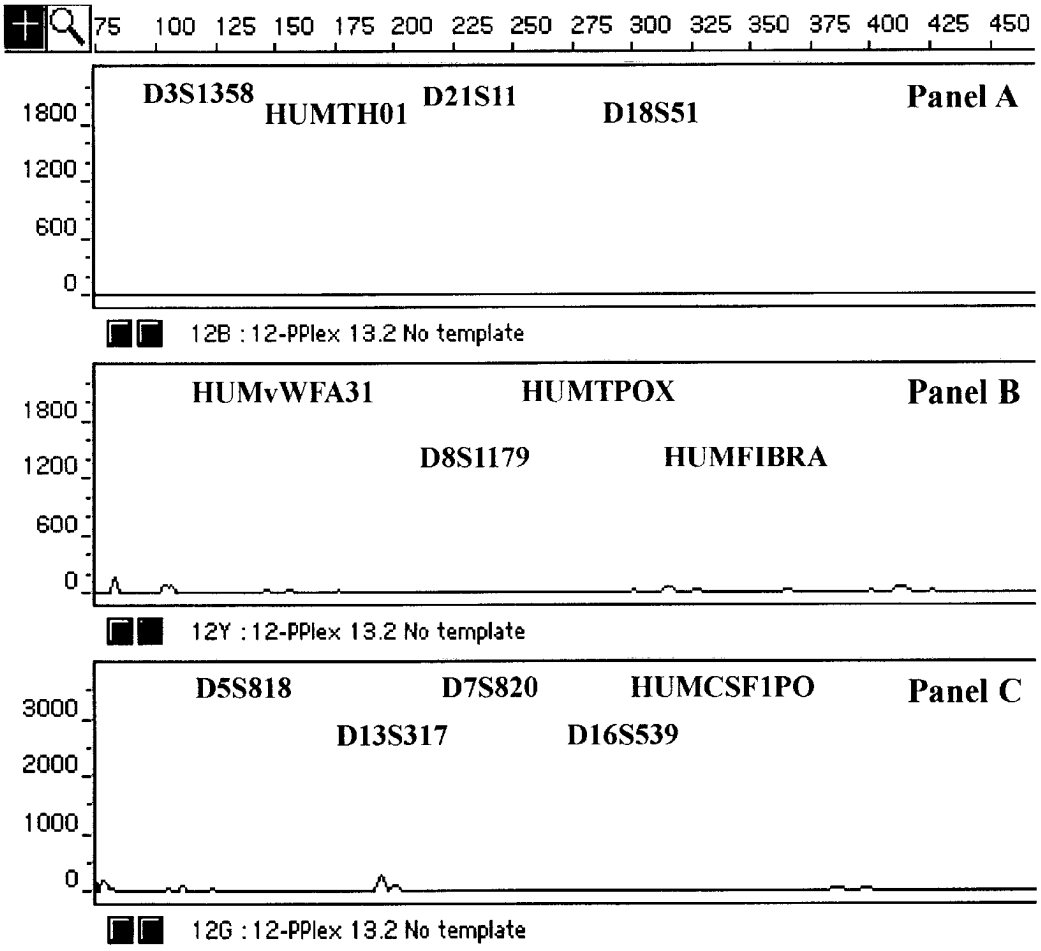


FIG. 2A

Amplification with DNA template

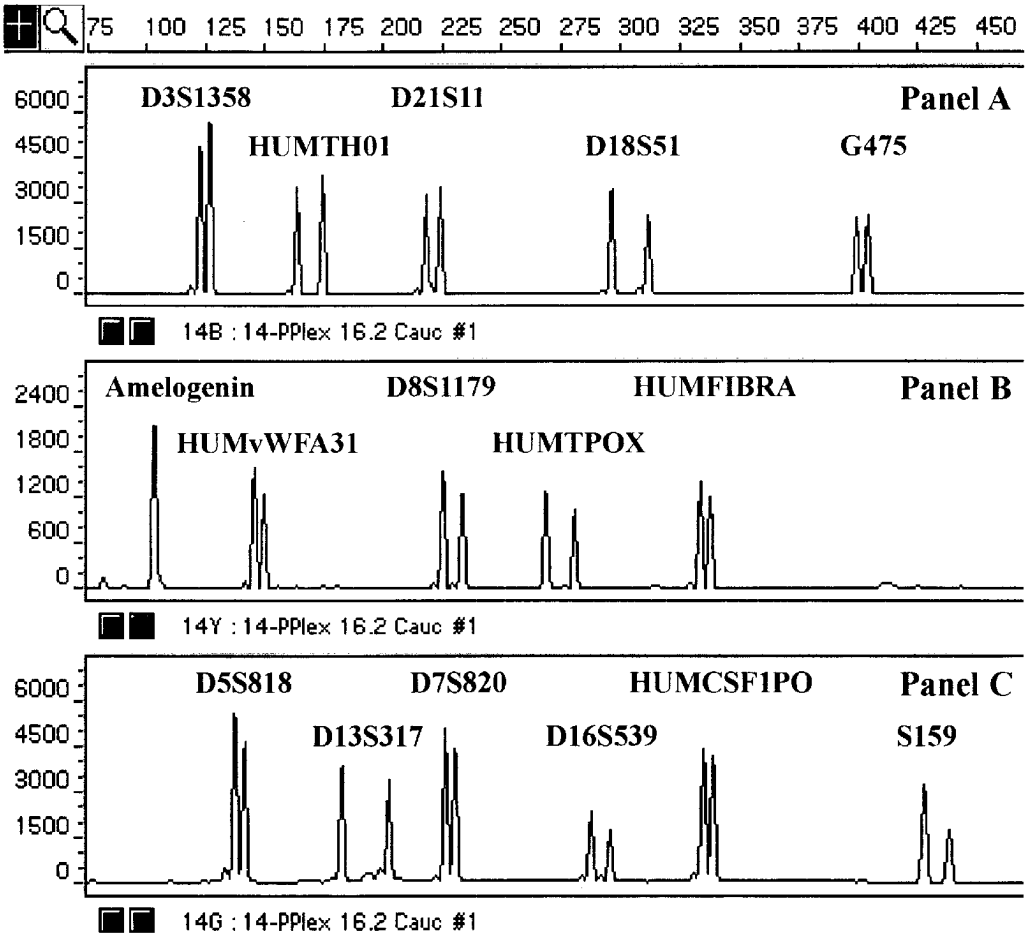


FIG. 2B

Amplification with NO DNA template

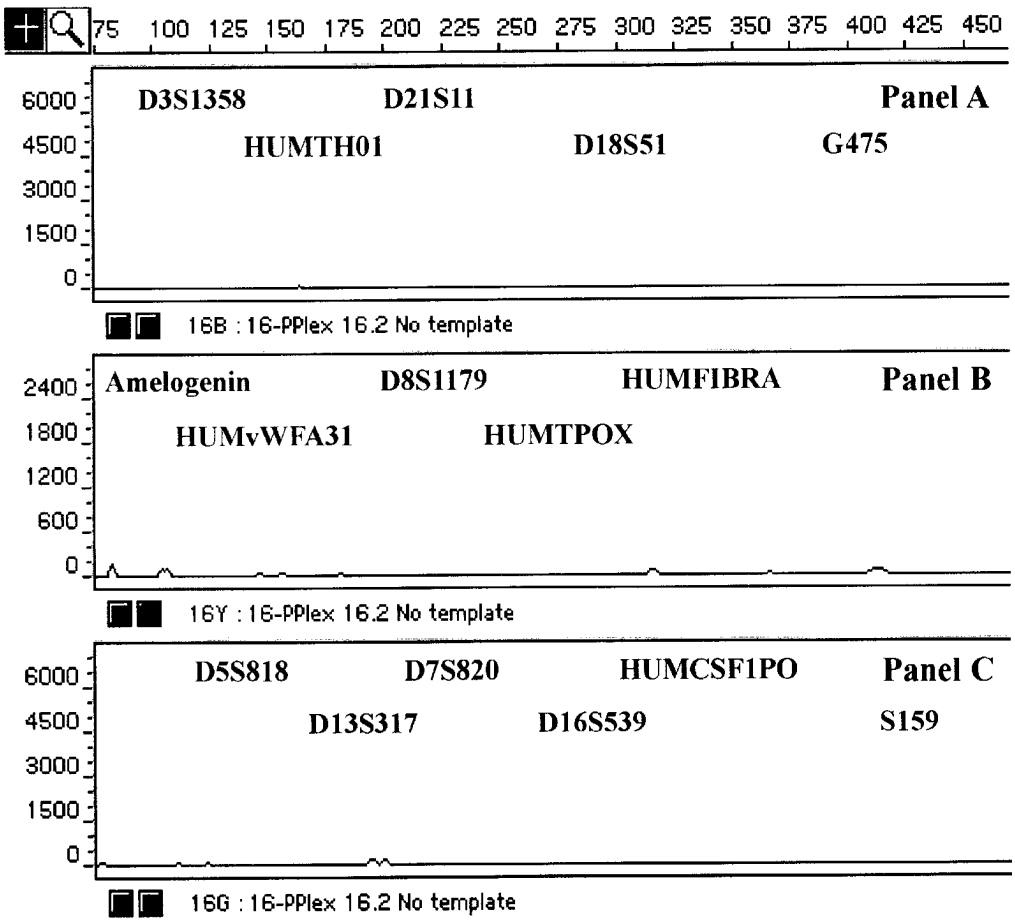


FIG. 3A

Amplification with DNA template

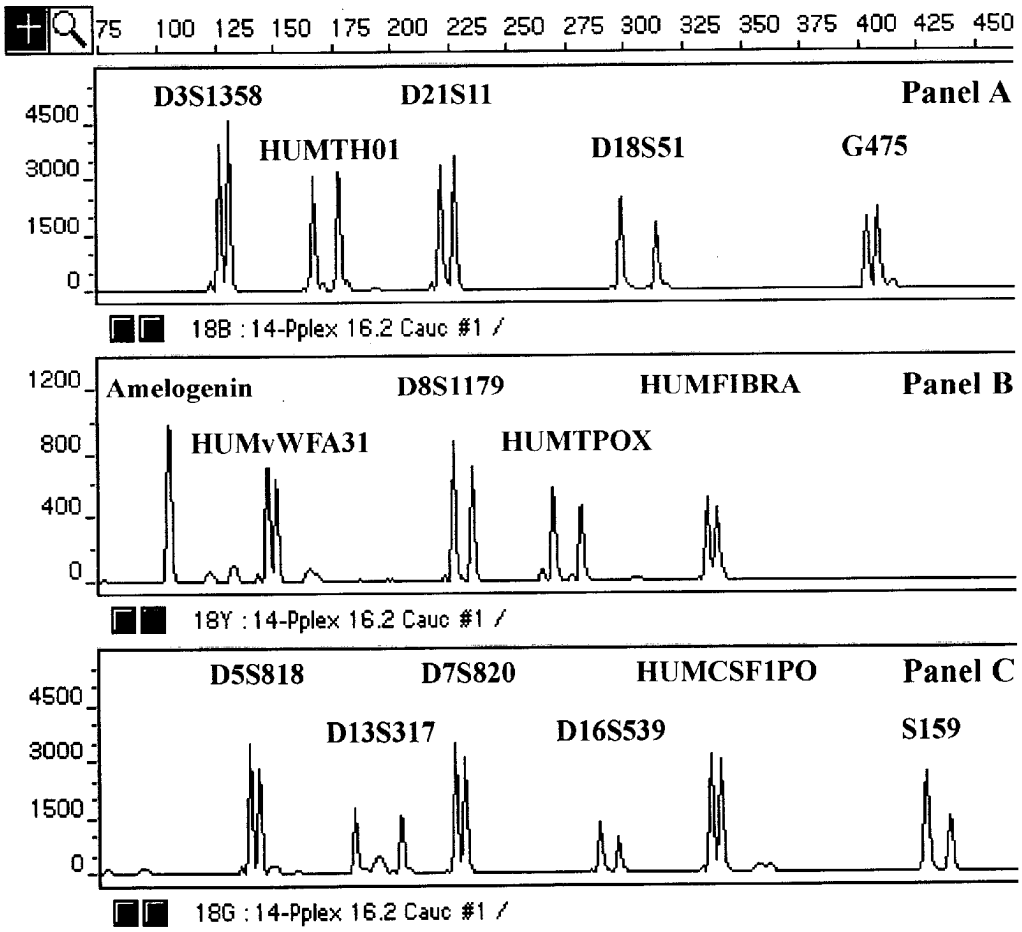


FIG. 3B

Amplification with NO DNA template

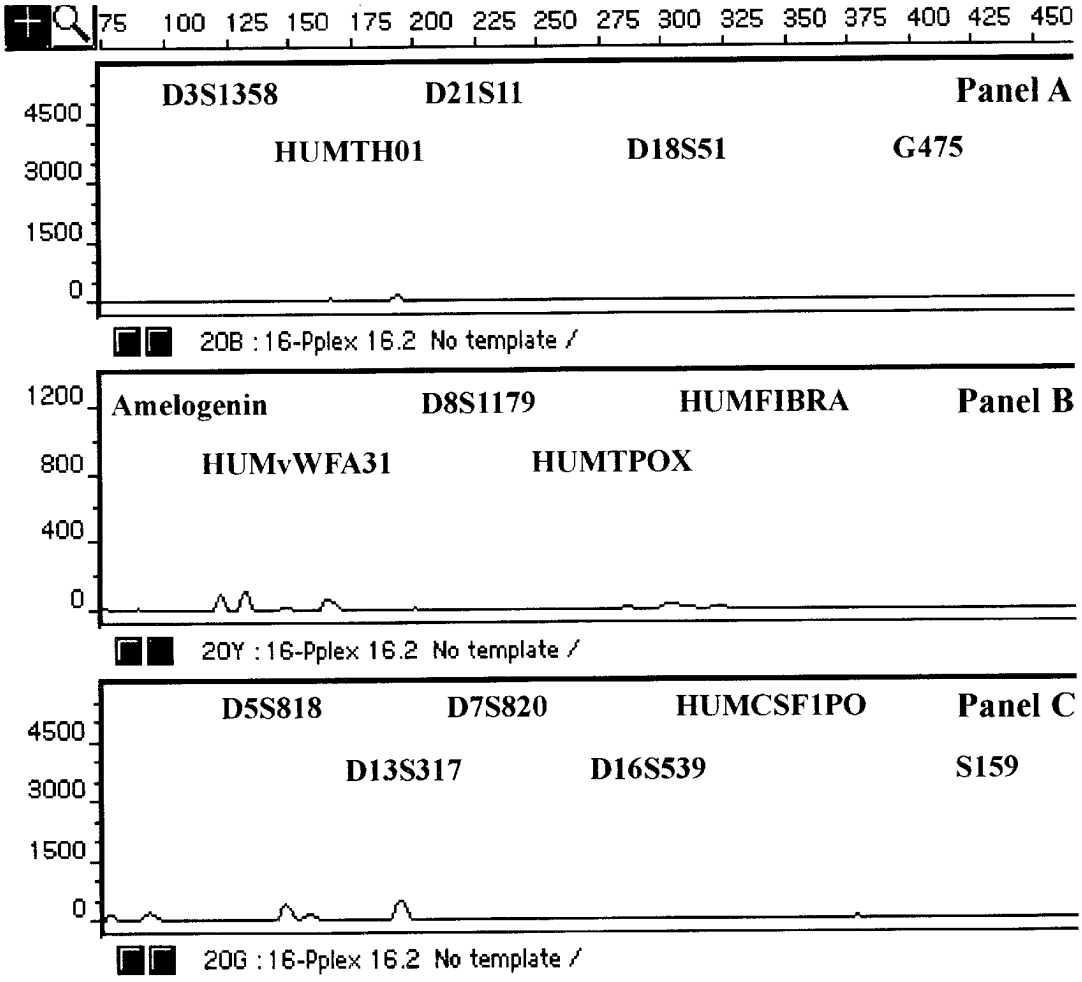


FIG 4A

505nm Scan: Fluorescein Channel

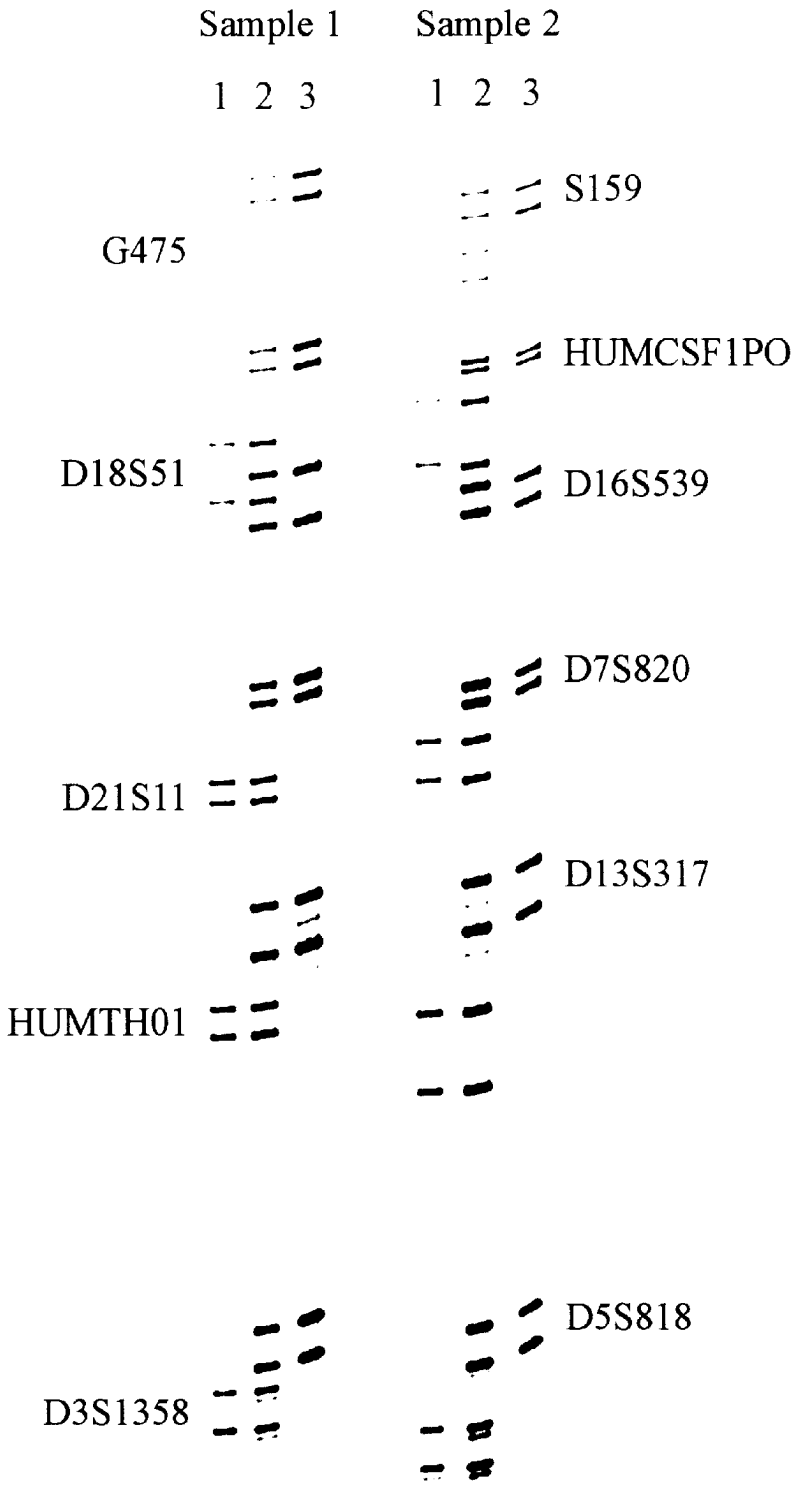


FIG 4B

585nm Scan: Tetramethyl Rhodamine Channel

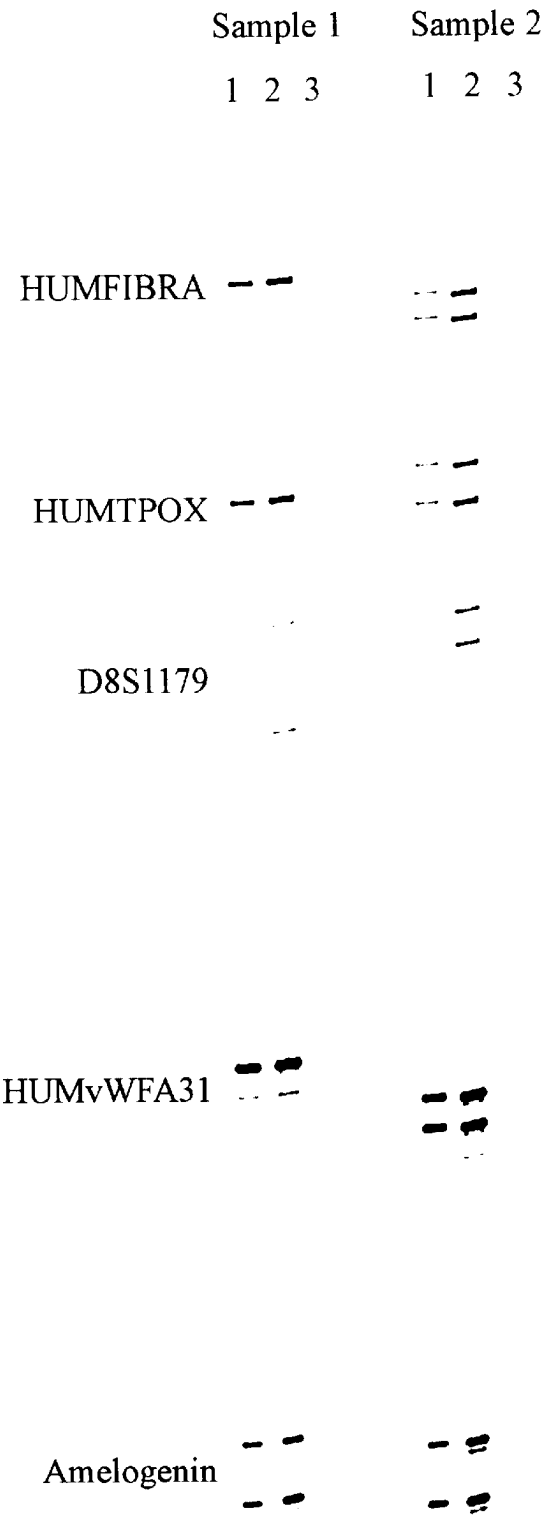


FIG 5A

505nm Scan: Fluorescein Channel

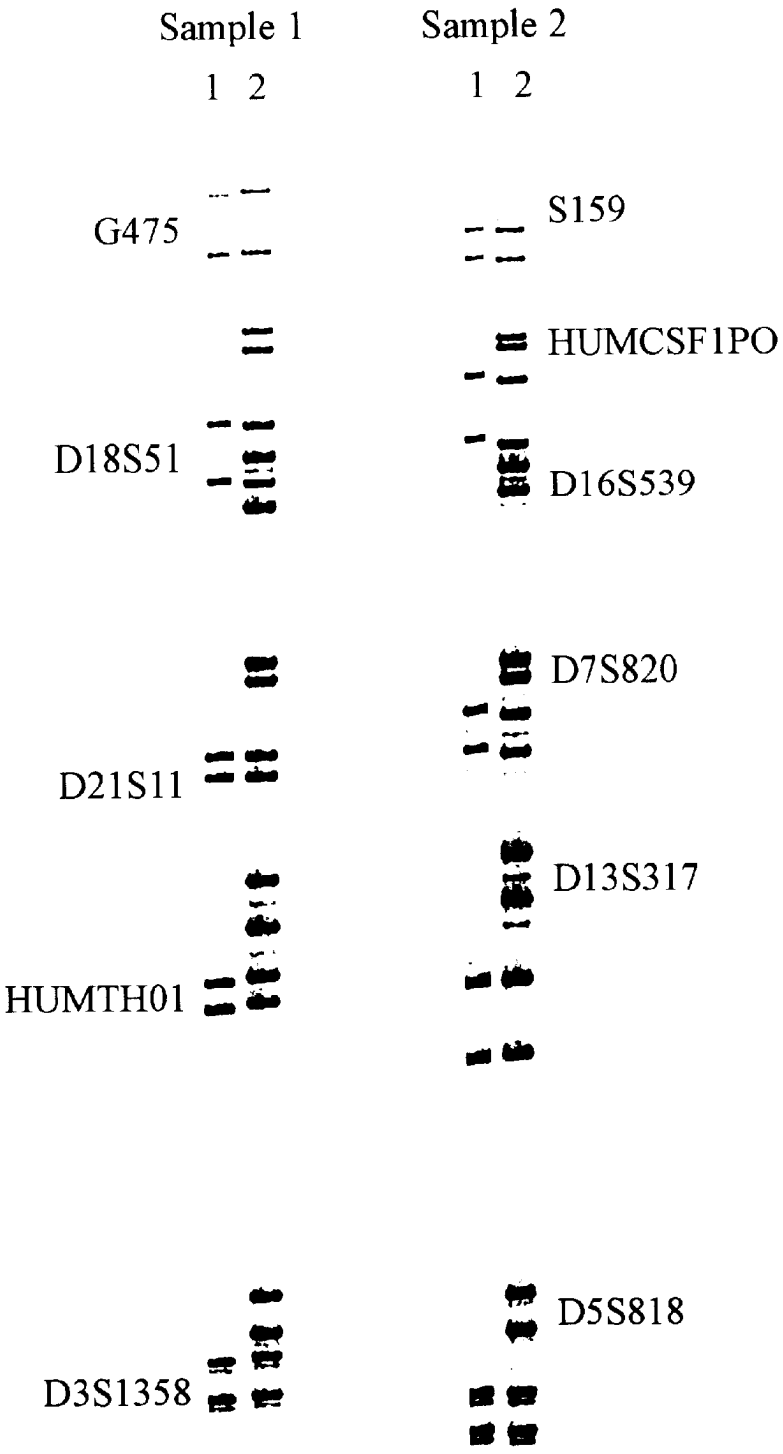


FIG 5B

585nm Scan: Tetramethyl Rhodamine Channel

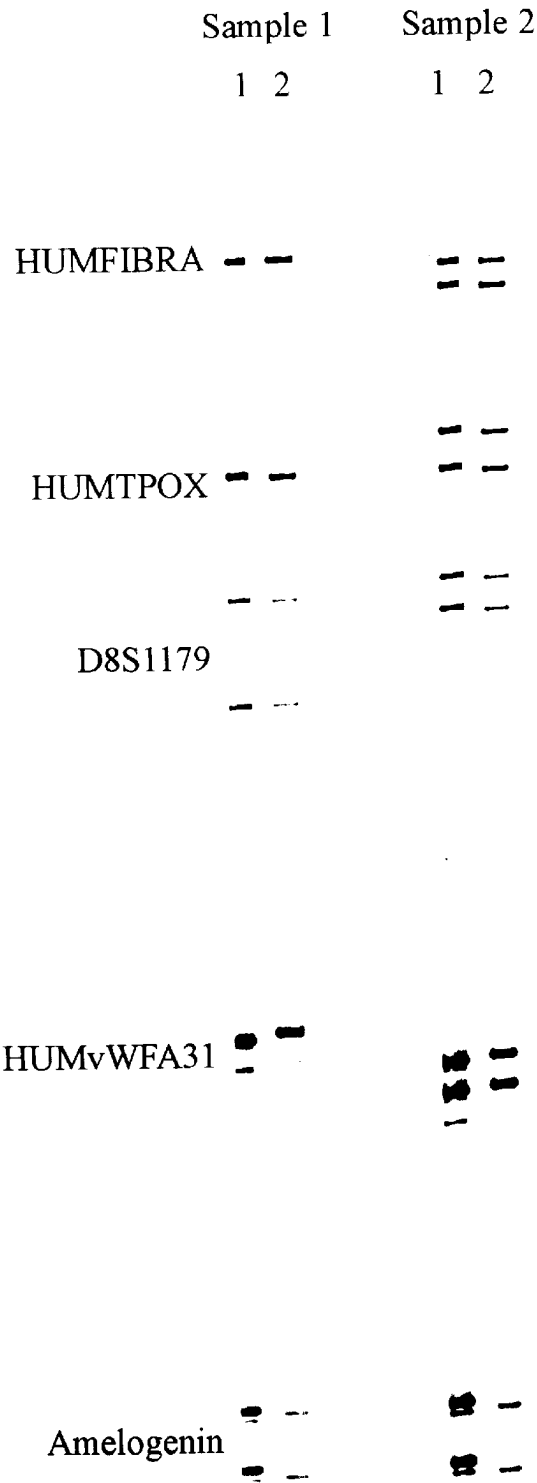


FIG 6A

505nm Scan: Fluorescein Channel

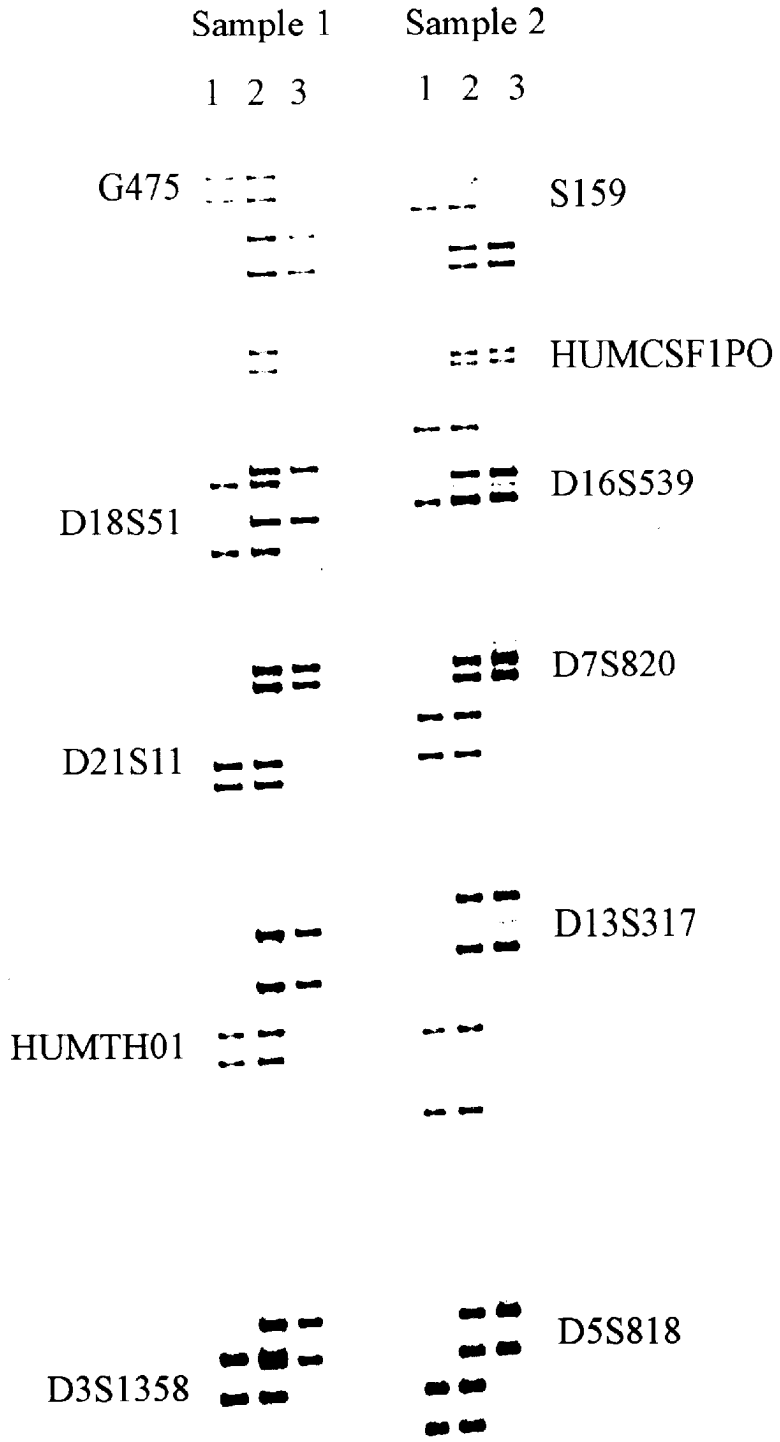
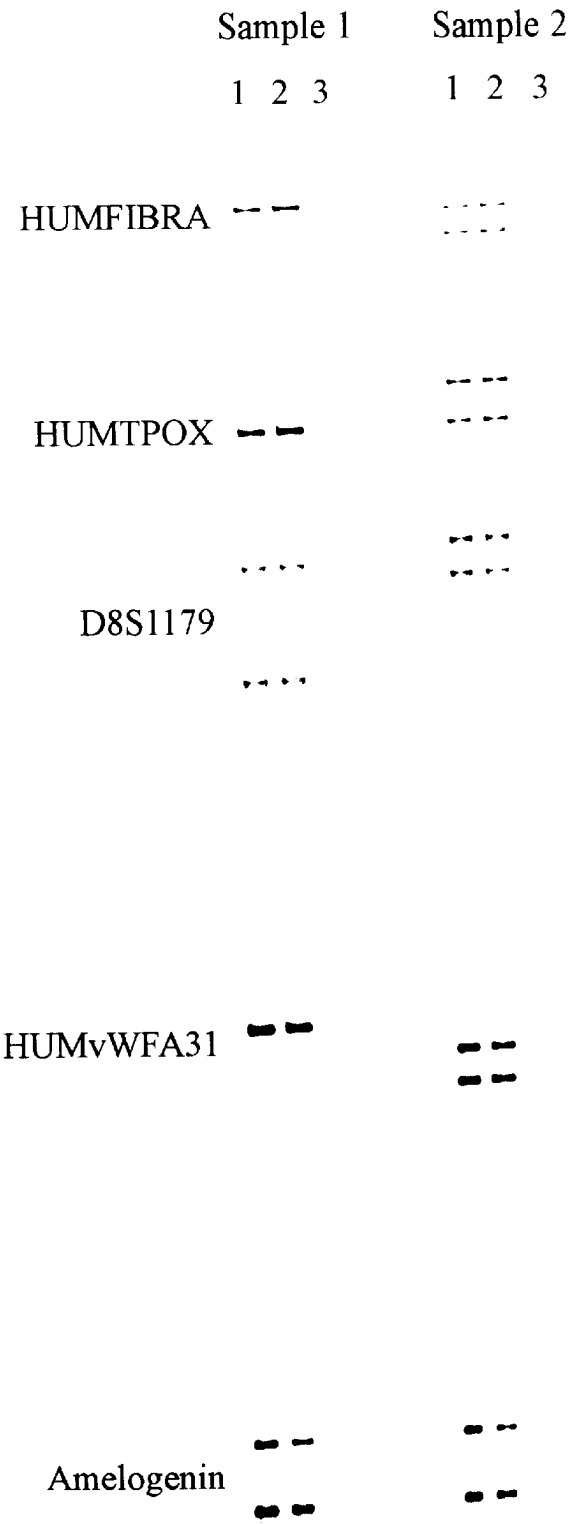


FIG 6B

585nm Scan: Tetramethyl Rhodamine Channel



MULTIPLEX AMPLIFICATION OF SHORT TANDEM REPEAT LOCI

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 08/632,575, filed Apr. 15, 1996, now U.S. Pat. No. 5,843,660, issued Dec. 1, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/316,544, filed Sep. 30, 1994. The entire disclosure of those applications is incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

FIELD OF THE INVENTION

The present invention is generally directed to the detection of genetic markers in a genomic system. The present invention is more specifically directed to the simultaneous amplification of multiple distinct polymorphic genetic loci using the polymerase chain reaction or other amplification systems to determine, in one reaction, the alleles of each locus contained within the multiplex system.

BACKGROUND OF THE INVENTION

DNA typing is commonly used to identify the parentage of human children, and to confirm the lineage of horses, dogs, other animals, and agricultural crops. DNA typing is also commonly employed to identify the source of blood, saliva, semen, and other tissue found at a crime scenes or other sites requiring identification of human remains. DNA typing is also employed in clinical settings to determine success or failure of bone marrow transplantation and presence of particular cancerous tissues. DNA typing involves the analysis of alleles of genomic DNA with characteristics of interest, commonly referred to as "markers". Most typing methods in use today are specifically designed to detect and analyze differences in the length and/or sequence of one or more regions of DNA markers known to appear in at least two different forms in a population. Such length and/or sequence variation is referred to as "polymorphism." Any region (i.e. "locus") of DNA in which such a variation occurs is referred to as a "polymorphic locus." The methods and materials of the present invention are designed for use in the detection of multiple loci of DNA, some or all of which are polymorphic loci.

Genetic markers which are sufficiently polymorphic with respect to length or sequence have long been sought for use in identity applications, such as paternity testing and identification of tissue samples collected for forensic analysis. The discovery and development of such markers and methods for analyzing such markers have gone through several phases of development over the last several years.

The first identified DNA variant markers were simple base substitutions, i.e. simple sequence polymorphisms, which were most often detected by Southern hybridization assays. For examples of references describing the identification of such markers, designed to be used to analyze restriction endonuclease-digested DNA with radioactive probes, see: Southern, E. M. (1975), *J. Mol. Biol.* 98(3):503–507; Schumm, et al. (1988), *American Journal of Human Genetics* 42:143–159; and Wyman, A. and White, R. (1980) *Proc. Natl. Acad. Sci, U.S.A.* 77:6754–6758.

The next generation of markers were size variants, i.e. length polymorphisms, specifically "variable number of tandem repeat" (VNTR) markers (Nakamura Y., et al. (1987), *Science* 235: 1616–1622; and U.S. Pat. No. 4,963,663 issued to White et al. (1990); U.S. Pat. No. 5,411,859 continuation of 4,963,663 issued to White et al. (1995)) and "minisatellite" markers (Jeffreys et al. (1985a), *Nature* 314:67–73; Jeffreys et al. (1985b) *Nature* 316:76–79., U.S. Pat. No. 5,175,082 for an invention by Jeffreys). Both VNTR and minisatellite markers, contain regions of nearly identical sequences repeated in tandem fashion. The core repeat sequence is 10 to 70 bases in length, with shorter core repeat sequences referred to as "minisatellite" repeats and longer repeats referred to as VNTRs. Different individuals in a human population contain different numbers of the repeats. The VNTR markers are generally more highly polymorphic than base substitution polymorphisms, sometimes displaying up to forty or more alleles at a single genetic locus. However, the tedious process of restriction enzyme digestion and subsequent Southern hybridization analysis are still required to detect and analyze most such markers.

The next advance involved the joining of the polymerase chain reaction (PCR) (U.S. Pat. No. 4,683,202 by Mullis, K. B.) technology with the analysis of VNTR loci (Kasai, K. et al. (1990) *Journal Forensic Science* 35(5):1196–1200). Amplifiable VNTR loci were discovered, which could be detected without the need for Southern transfer. The amplified products are separated through agarose or polyacrylamide gels and detected by incorporation of radioactivity during the amplification or by post-staining with silver or ethidium bromide. However, PCR can only be used to amplify relatively small DNA segments reliably, i.e. only reliably amplifying DNA segments under 3,000 bases in length Ponce, M & Micol, L. (1992) NAR 20(3):623; Decorte R, et al. (1990) *DNA Cell Biol.* 9(6):461–469). Consequently, very few amplifiable VNTRs have been developed.

In recent years, the discovery and development of polymorphic short tandem repeats (STRs) as genetic markers has stimulated progress in the development of linkage maps, the identification and characterization of diseased genes, and the simplification and precision of DNA typing. Specifically, with the discovery and development of polymorphic markers containing dinucleotide repeats (Litt and Luty (1989) *Am J. Hum Genet* 3(4):599–605; Tautz, D (1989) *NAR* 17:6463–6471; Weber and May (1989) *Am J Hum Genet* 44:388–396; German Pat. No. DE 38 34 636 C2, inventor Tautz, D; U.S. Pat. No. 5,582,979 filed by Weber, L.), STRs with repeat units of three to four nucleotides (Edwards, A., et al. (1991) *Am. J. Hum. Genet.* 49: 746–756.; Hammond, H. A., et al. (1994) *Am. J. Hum. Genet.* 55: 175–189; Fregeau, C. J.; and Fourney, R. M. (1993) *BioTechniques* 15(1): 100–119.; Schumm, J. W. et al. (1994) in *The Fourth International Symposium on Human Identification* 1993, pp. 177–187 (pub. by Promega Corp., 1994); and U.S. Pat. No. 5,364,759 by Caskey et al.; German Pat. No. DE 38 34 636 C2 by Tautz, D.) and STRs with repeat units of five to seven bases (See, e.g. Edwards et al. (1991) *Nucleic Acids Res.* 19:4791; Chen et al. (1993) *Genomics* 15(3): 621–5; Harada et al. (1994) *Am. J. Hum. Genet.* 55: 175–189; Comings et al. (1995), *Genomics* 29(2):390–6; and Utah Marker Development Group (1995), *Am. J. Genet.* 57:619–628; and Jurka and Pethiyagoda (1995) *J. Mol. Evol.* 40:120–126)), many of the deficiencies of previous methods have been overcome. STR markers are generally shorter than VNTR markers, making them better substrates for amplification than most VNTR markers.

STR loci are similar to amplifiable VNTR loci in that the amplified alleles at each such locus may be differentiated

based on length variation. Generally speaking STR loci are less polymorphic at each individual locus than VNTR loci. Thus, it is desirable to amplify and detect multiple STR systems in a single amplification reaction and separation to provide information for several loci simultaneously. Systems containing several loci are called multiplex systems and many such systems containing up to 11 separate STR loci have been described. See, e.g., *Proceedings: American Academy of Forensic Sciences* (Feb. 9–14, 1998), Schumm, James W. et al., p. 53, B88; Id., Gibson, Sandra D. et al., p. 53, B89; Id., Lazaruk, Katherine et al., p. 51, B83; Sparkes, R. et al., *Int J Legal Med* (1996) 109:186–194; *AmpFlSTR Profiler™ PCR Amplification Kit User's Manual* (1997), pub by Perkin-Elmer Corp, i-viii and 1-1 to 1-10; *AmpFlSTR Profiler Plus™ PCR Amplification Kit User's Manual* (1997), pub by Perkin-Elmer Corp., i viii and 1-1 to 1-10; *AmpFlSTR COfiler™ PCR Amplification Kit User Bulletin* (1998), pub by Perkin-Elmer Corp. i-iii and 1-1 to 1-10; *9th International Symposium on Human Identification* (Oct. 7–10, 1998), pub. by Promega Corp., Staub, Rick W. et al., Poster Abstract 15; Id., Willard, Jeanne M. et al., Poster Abstract 73; and Id., Walsh, P. Sean, et al., Speaker Abstract for 8:50am–9:20am, Thursday, Oct. 8, 1998.

Amplification protocols with STR loci can be designed to produce small products, generally from 60 to 500 base pairs (bp) in length, and alleles from each locus are often contained within a range of less than 100 bp. This allows simultaneous electrophoretic analysis of several systems on the same gel or capillary electrophoresis by careful design of PCR primers such that all potential amplification products from an individual system do not overlap the range of alleles of other systems. Design of these systems is limited, in part, by the difficulty in separating multiple loci in a single gel or capillary. This occurs because there is spacial compression of fragments of different sizes, especially longer fragments in gels or capillaries, i.e., commonly used means for separation of DNA fragments by those skilled in the art.

The United States Federal Bureau of Investigation ("FBI") has established and maintains a Combined DNA Index System ("CODIS"), a database of DNA typing information. Local, state, and national law enforcement agencies use the CODIS system to match forensic DNA evidence collected at crime scenes with DNA information in the database. CODIS and other national database systems have proven to be an effective tool for such agencies to use in solving violent crimes. (See, e.g. Niezgoda, Stephen, in Cambridge Healthtech Institute's Second Annual Conference on DNA Forensics: Science, Evidence, and Future Prospects (Nov. 17–18, 1998), pp. 1–21.; Niezgoda, Stephen in *Proceedings From The Eighth International Symposium on Human Identification* 1997, pub. by Promega Corporation (1998), pp 48–49; Frazier, Rachel R. E. et al. Id., pp. 56–60; Niezgoda, S. J. *Profiles in DNA* 1(3): 12–13; Werrett, D. J. and Sparkes, R. in *Speaker Abstracts: 9th International Symposium on Human Identification* (Oct. 7–10, 1998) pp. 5–6). Until recently, only restriction fragment length polymorphism ("RFLP") data obtained from the analysis of particular VNTR loci was considered a core component in the database. The FBI has recently identified thirteen polymorphic STR loci for inclusion in the CODIS database. The thirteen CODIS STR loci are HUMCSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31. (Budowle, Bruce and Moretti, Tamyra in *Speaker Abstracts: 9th International Symposium on Human Identification* (Oct. 7–10, 1998) pp. 7–8). Both VNTR and STR marker data are currently maintained in the CODIS database. (See, e.g. Niezgoda, Stephen in Second Annual Conference on DNA Forensics, supra). Until the present invention, the number of loci which could be co-amplified in a single reaction, and analyzed thereafter was limited. Specifically, no materials or methods had been developed for use in multiplex amplification of thirteen or more STR loci, much less the thirteen polymorphic STR loci identified for use in the CODIS database.

The materials and methods of the present method are designed for use in multiplex analysis of particular polymorphic loci of DNA of various types, including single-stranded and double-stranded DNA from a variety of different sources. The present invention represents a significant improvement over existing technology, bringing increased power of discrimination, precision, and throughput to DNA profiling for linkage analysis, criminal justice, paternity testing, and other forensic, medical, and genetic identification applications.

SUMMARY OF THE INVENTION

It is, therefore, an object of the present invention to provide a method and materials for the simultaneous amplification of sets of loci, which include multiple distinct polymorphic short tandem repeat (STR) loci, in a single multiplex reaction, using PCR or other amplification systems in combination with gel electrophoresis, capillary electrophoresis or other separation and detection methods to analyze and compare the relative lengths of the alleles of each locus amplified in the multiplex reaction. Multiplex analysis of the sets of loci disclosed herein has not been previously described in the prior art. There has also not been any previous description of the sequences for many of the primers disclosed herein below, all of which are shown to be useful in multiplex amplification of the sets of loci disclosed.

It is also an object of the present invention to provide a method, a kit, and primers specific for multiplex amplifications comprising specified loci.

These and other objects are addressed by the present invention which is directed to a method and materials for simultaneously analyzing or determining the alleles present at each individual locus of each multiplex. In general, the method of this invention comprises the steps of (a) obtaining at least one DNA sample to be analyzed, wherein the DNA sample has at least thirteen loci which can be co-amplified; (b) co-amplifying the at least thirteen loci of the DNA sample; and (c) detecting the amplified materials in a fashion which reveals the polymorphic nature of the systems employed.

In one embodiment, the present invention is a method of simultaneously determining the alleles present in a set of loci from one or more DNA samples, comprising the steps of:

(a) obtaining at least one DNA sample to be analyzed;

(b) selecting a set of loci of the DNA sample, comprising at least thirteen short tandem repeat loci which can be co-amplified;

(c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

At least four of the at least thirteen short tandem repeat loci are preferably selected from the group of loci consisting of:

D3S1539, D4S2368, D5S818, D7S820, D9S930, D10S1239, D13S317, D14S118, D14S548, D14S562, D16S490, D16S539, D16S753, D17S1298, D17S1299, D19S253, D20S481, D22S683, HUMCSF1PO, HUMTPOX, HUMTH01, HUMF13A01, HUMBFXIII, HUMLIPOL, HUMvWFA31.

In another embodiment of the invention, the set of loci selected in step (b) of In another embodiment of the invention, the set of loci selected in step (b) of the method comprises thirteen CODIS STR loci (i.e., D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31) which can be co-amplified and analyzed by themselves, or with additional loci using methods of the present invention.

In a further aspect, this invention is a kit for simultaneously analyzing a set of loci of genomic DNA, comprising oligonucleotide primers for co-amplifying a set of loci of the genomic DNA to be analyzed, wherein the set of loci comprises at least thirteen short tandem repeat loci which can be co-amplified in the same multiplex reaction, and wherein the primers are in one or more containers. More preferably, the kit comprises oligonucleotide primer pairs for co-amplifying a set of at least thirteen loci of human genomic DNA, the set of loci comprising D3S1358, D5S818, D7S820, D8S1179, D 13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31.

In yet a further aspect, the invention is primer sequences and primer pairs for amplifying specific loci of human DNA. Use of the primers and primer pairs of this invention for multiplex analysis of human DNA is demonstrated herein, below. The primers of this invention are suitable for use in the method of this invention, wherein they can be used in labeled form, as noted below, to assist the evaluation step of the method.

The approaches specified in the present invention produce savings of time, labor, and materials in the analysis of loci contained within the multiplexes. The method of the present invention allows thirteen or more, even as many as sixteen or more, loci to be co-amplified in one tube using a single amplification reaction, instead of amplifying each locus independently in separate tubes or in smaller groups of loci.

The present invention has specific use in the field of forensic analysis, paternity determination, monitoring of bone marrow transplantation, linkage mapping, and detection of genetic diseases and cancers. By allowing thirteen methods of the present invention significantly increase the certainty with which one can match DNA prepared from different samples from the same individual. The need to match or distinguish accurately between samples containing very small amounts of DNA is particularly acute in forensics applications, where many convictions (and acquittals) turn on DNA typing analysis.

Scientists, particularly forensic scientists, have long appreciated the need to analyze multiple polymorphic loci of DNA in order to ensure that a match between two samples of DNA is statistically significant. (Presley, L. A. et al., in *The Third International Symposium on Human Identification* 1992, pp. 245–269 (pub. by Promega Corp., 1993); Bever, R. A., et al., in *The Second International Symposium on Human Identification* 1991, pp.103–128. (pub. by Promega Corp., 1992)). However, until this invention, one could not simultaneously analyze thirteen or more STR loci in a single reaction. To realize the importance of such multiplexing capabilities, it helps to understand some of the mathematics behind DNA typing analysis.

For purposes of illustration, suppose every STR locus has a genotype (i.e., pattern of two alleles) frequency of one in ten. In other words, suppose that the chance of two randomly selected individuals have a matching type for a single STR is 1/10. However, if two different STR loci are analyzed, the chance of a random match with both systems is 1/100. If three STR loci are analyzed, the chances of a random match with each of the three systems is 1/1,000 and so on. Consequently, it is easy to see how increasing the number of STR loci analyzed reduces the likelihood of random matches within the general population, thereby increasing the chance one can accurately identify a suspect's presence at a crime scene by comparing the individual's type with crime scene evidence. Similar reasoning can be used to conclude that the method of this invention also would increase the likelihood of accurately identifying a suspected father in a paternity case, of correctly matching bone marrow tissue, of developing significant results from linkage mapping studies, and of detecting genetic diseases and cancers.

Further objects, features, and advantages of the invention will be apparent from the following best mode for carrying out the invention and the illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A is a plot of the output of three-color fluorescent detection of the products of simultaneous amplification of the loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31 of a sample of human genomic DNA, as detected with the ABI PRISM® 310 Genetic Analyzer in Example 1.

FIG. **1**B is a plot of the output of three-color fluorescent detection of a control sample processed the same way as FIG. **1**A, with no genomic DNA in the amplification reaction.

FIG. **2**A is a plot of the output of three-color fluorescent detection of the products of simultaneous amplification of the loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21 S11, HUMCSF1PO HUMFIBRA, HUMTH01, HUMTPOX, HUMvWFA31, G475, S159, and Amelogenin of a sample of human genomic DNA, as detected with the ABI PRISM® 310 Genetic Analyzer in Example 2.

FIG. **2**B is a plot of the output of three-color fluorescent detection of a control sample processed the same way as FIG. **2**A, with no genomic DNA substrate in the amplification reaction.

FIG. **3**A is a plot of the output of three-color fluorescent detection of the products of simultaneous amplification of the loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, HUMvWFA31, G475, S159, and Amelogenin of a sample of human genomic DNA, as detected with an ABI PRISM® 377 DNA Sequencer in Example 3.

FIG. **3**B is a plot of the output of three-color fluorescent detection of a control sample processed the same way as FIG. **3**A, with no genomic DNA substrate in the amplification reaction.

FIGS. **4**A and **4**B are laser printed images of the results of fluorescent detection of the products of simultaneous amplification of the loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, HUMvWFA31, G475, S159, and Amelogenin as detected using the fluorescein channel (FIG. **4**A) and carboxy-tetramethylrhodamine

channel (FIG. **4**B) of a Hitachi FMBIO® II Fluorescent Scanner, as described in Example 4.

FIGS. **5**A and **5**B are laser printed images of the results of fluorescent detection of the products of simultaneous amplification of the loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, HUMvWFA31, G475, S159, and Amelogenin as detected using the fluorescein channel (FIG. **5**A) and carboxy-tetramethylrhodamine channel (FIG. **5**B) of a Hitachi FMBIO® II Fluorescent Scanner, as described in Example 5.

FIGS. **6**A and **6**B are laser printed images of the results of fluorescent detection of the products of simultaneous amplification of the loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, HUMvWFA31, C221, S159, and Amelogenin as detected using the fluorescein channel (FIG. **6**A) and carboxy-tetramethylrhodamine channel (FIG. **6**B) of a Hitachi FMBIO® II Fluorescent Scanner, as described in Example 6.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

The following definitions are intended to assist in providing a clear and consistent understanding of the scope and detail of the following terms, as used to describe and define the present invention:

"Allelic ladder": a standard size marker consisting of amplified alleles from the locus.

"Allele": a genetic variation associated with a segment of DNA, i.e., one of two or more alternate forms of a DNA sequence occupying the same locus.

"Biochemical nomenclature": standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and Cytosine (C). Corresponding nucleotides are, for example, deoxyguanosine-5'-triphosphate (dGTP).

"DNA polymorphism": the condition in which two or more different nucleotide sequences in a DNA sequence coexist in the same interbreeding population.

"Locus" or "genetic locus": a specific position on a chromosome. Alleles of a locus are located at identical sites on homologous chromosomes.

"Locus-specific primer": a primer that specifically hybridizes with a portion of the stated locus or its complementary strand, at least for one allele of the locus, and does not hybridize efficiently with other DNA sequences under the conditions used in the amplification method.

"Pentanucleotide tandem repeat": a subclass of the STR polymorphisms defined below. Unless specified otherwise, the term "pentanucleotide tandem repeat" encompasses perfect STRs wherein the repeat unit is a five base sequence, and imperfect STRs wherein at least one repeat unit is a five base repeat.

"Polymerase chain reaction" or "PCR": a technique in which cycles of denaturation, annealing with primer, and extension with DNA polymerase are used to amplify the number of copies of a target DNA sequence by approximately 10^6 times or more. The polymerase chain reaction process for amplifying nucleic acid is covered by U.S. Pat. Nos. 4,683,195 and 4,683,202, which are incorporated herein by reference for a description of the process.

"Polymorphic short tandem repeat loci": STR loci, defined below, in which the number of repetitive sequence elements (and net length of sequence) in a particular region of genomic DNA varies from allele to allele, and from individual to individual.

"Polymorphism information content" or "PIC": a measure of the amount of polymorphism present at a locus (Botstein et al., 1980). PIC values range from 0 to 1.0, with higher values indicating greater degrees of polymorphism. This measure generally displays smaller values than the other commonly used measure, i.e., heterozygosity. For markers that are highly informative (heterozygosities exceeding about 70%), the difference between heterozygosity and PIC is slight.

"Primer": a single-stranded oligonucleotide or DNA fragment which hybridizes with a DNA strand of a locus in such a manner that the 3' terminus of the primer may act as a site of polymerization using a DNA polymerase enzyme.

"Primer pair": two primers including, primer 1 that hybridizes to a single strand at one end of the DNA sequence to be amplified and primer 2 that hybridizes with the other end on the complementary strand of the DNA sequence to be amplified.

"Primer site": the area of the target DNA to which a primer hybridizes.

"Short tandem repeat loci" or "STR loci": regions of genomic DNA which contain short, repetitive sequence elements of 3 to 7 base pairs in length. The term STR also encompasses a region of genomic DNA wherein more than a single three to seven base sequence is repeated in tandem or with intervening bases, provided that at least one of the sequences is repeated at least two times in tandem. Each sequence repeated at least once within an STR is referred to herein as a "repeat unit."

The sequences of the STR loci analyzed using the materials and methods of the present invention can be divided into two general categories, perfect and imperfect. The term "perfect" STR, as used herein, refers to a region of double-stranded DNA containing a single three to seven base repeat unit repeated in tandem at least two times, e.g. $(AAAAT)_2$. The term "imperfect" STR, as used herein, refers to a region of DNA containing at least two tandem repeats of a perfect repeat unit and at least one repeat of an imperfect repeat unit, wherein the imperfect repeat unit consists of a DNA sequence which could result from one, two, three, or four base insertions, deletions, or substitutions in the sequence of the perfect repeat unit, e.g. $(AAAAT)_{12}(AAAAAT)_5AAT(AAATT)_4$. Every imperfect STR sequence contains at least one perfect STR sequence. Specifically, every STR sequence, whether perfect or imperfect, includes at least one repeat unit sequence appearing at least two times in tandem, a repeat unit sequence which can be represented by formula (I):

$$(A_wG_xT_yC_z)_n \quad (I)$$

wherein A, G, T, and C represent the nucleotides which can be in any order; w, x, y and z represent the number of each nucleotide in the sequence and range from 0 to 7 with the sum of w+x+y+z ranging between 3 and 7; and n represents the number of times the sequence is tandemly repeated and is at least 2.

B. Selection of Multiplex Reaction Components

The method of the present invention contemplates selecting an appropriate set of loci, primers, and amplification protocols to generate amplified alleles from multiple co-amplified loci which preferably do not overlap in size or, more preferably, which are labeled in a way which enables one to differentiate between the alleles from different loci which overlap in size. In addition, this method contemplates the selection of short tandem repeat loci which are compatible for use with a single amplification protocol. The specific combinations of loci described herein are unique in this

application. Combinations of loci may be rejected for either of the above two reasons, or because, in combination, one or more of the loci do not produce adequate product yield, or fragments which do not represent authentic alleles are produced in this reaction.

Successful combinations in addition to those disclosed herein can be generated by trial and error of locus combinations, by selection of primer pair sequences, and by adjustment of primer concentrations to identify an equilibrium in which all included loci may be amplified. Once the method and materials of this invention are disclosed, various methods of selecting loci, primer pairs, and amplification techniques for use in the method and kit of this invention are likely to be suggested to one skilled in the art. All such methods are intended to be within the scope of the appended claims.

Of particular importance in the practice of the method of this invention is the size range of amplified alleles produced from the individual loci which are co-amplified in the multiplex amplification reaction step. For ease of analysis with current technologies, systems which can be detected by amplification of fragments smaller than 500 bases are most preferable.

Practice of the method of the present invention begins with selection of a set of loci comprising at least thirteen STR loci, which can be co-amplified in a single multiplex amplification reaction. Selection of loci and oligonucleotide primers used to amplify the loci in the multiplex amplification reaction of the present method is described herein below, and illustrated in the Examples below.

C. Use of Multiplexes of Three Loci to Develop Multiplexes Using More than Three Loci

Any one of a number of different techniques can be used to select a set of loci for use in the present invention. One preferred technique for developing useful sets of loci for use in this method of analysis is described below. Once a multiplex containing three STR loci is developed, it may be used as a core to create multiplexes containing more than three loci. New combinations of more than three loci can, thus, be created which include the first three loci. For example, the core multiplex containing loci D7S820, D13S317, and D5S818 was used to generate derivative multiplexes of:

D16S539, D7S820, D13S317, and D5S818;

HUMCSF1PO, HUMTPOX, D16S539, D7S820, D13S317, and D5S818;

HUMCSF1PO, HUMTPOX, HUMTH01, D16S539, D7S820, D13S317, and D5S818;

HUMCSF1PO, HUMTPOX, HUMTH01, HUMvWFA31, D16S539, D7S820, D13S317, and D5S818;

D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWA31;

S159, HUMCSF1PO, D16S539, D7S820, D13S317, and D5S818;

D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31; and

D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, HUMvWFA31, G475, S159, and Amelogenin.

It is contemplated that core sets of loci can be used to generate other appropriate derivative sets of STR loci for multiplex analysis using the method of this invention. Regardless of what method is used to select the loci analyzed using the method of the present invention, all the loci selected for multiplex analysis share the following characteristics: (1) they produce sufficient amplification product to allow evaluation; (2) they generate few if any artifacts due to the addition (or lack of addition) of a base to the amplified alleles during the multiplex amplification step; (3) they generate few, if any, artifacts due to premature termination of amplification reactions by a polymerase; and (4) they produce little or no "trailing" bands of smaller molecular weight from consecutive single base deletions below a given authentic amplified allele. See, e.g., Schumm et al., *Fourth International Symposium on Human Identification* 1993, pp. 177–187 (pub. by Promega Corp., 1994).

The same technique used to identify the set of at least three loci, described above, can be applied to select thirteen or more loci of human genomic DNA or multiplex analysis, according to a preferred embodiment of the method of analysis of the present invention. Any set of loci identified as described above is suitable for multiplex analysis in accordance with the present invention, provided the set of loci comprises at least thirteen STR loci. More preferably, at least four of the at least thirteen STR loci analyzed according to the present invention are selected from the group of loci consisting of:

D3S1539, D4S2368, D5S818, D7S820, D9S930, D10S1239, D13S317, D14S118, D14S548, D14S562, D16S490, D16S539, D16S753, D17S1298, D17S1299, D19S253, D20S481, D22S683, HUMCSF1PO, HUMTPOX, HUMTH01, HUMF13A01, HUMBFXIII, HUMLIPOL, and HUMvWFA31

Even more preferably, the set of loci analyzed according to the present invention includes all thirteen CODIS loci, i.e. D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31.

At least one of the loci selected for co-amplification in the present multiplex reaction is preferably an STR locus with a repeat unit of five to seven bases or base pairs in length, more preferably an STR locus with a pentanucleotide repeat. As is demonstrated in U.S. patent application Ser. No. 09/018,584, which is incorporated by reference herein, loci with such intermediate length repeats can be amplified with minimal incidence of artifacts, e.g. due to repeat slippage. Three such loci with pentanucleotide repeats, G475, C221 and S159, are included in the sets of loci identified immediately above. The terms "G475", "C221 ", and "S159", as used herein, refer to names assigned to pentanucleotide repeat loci identified, as described in U.S. patent application Ser. No. 09/018,584, incorporated by reference above. Each name corresponds to a clone from which each pentanucleotide locus was identified. The sequence of the G475 clone, described therein as SEQ ID NO:34, is identified herein as SEQ ID NO:108. The sequence of the C221 clone, described therein as SEQ ID NO:2, is identified herein as SEQ ID NO:109. The sequence of the S159 clone, described therein as SEQ ID NO: 26, is identified herein as SEQ ID NO:110. Individual primers and primer pairs identified for use in amplifying G475, C221, and S159 therein can also be used to amplify the same loci in the sets of at least thirteen loci co-amplified and analyzed according to the present invention.

The set of loci selected for co-amplification and analysis according to the invention preferably further comprises at least one locus in addition to the at least thirteen STR loci. The additional locus preferably includes a sequence

polymorphism, or another feature which identifies a particular characteristic which separates the DNA of an individual from the DNA of other individuals in the population. The additional locus more preferably is a locus which identifies the gender of the source of the DNA sample analyzed. When the DNA sample is human genomic DNA, a gender identifying locus such as the Amelogenin locus is preferably selected for co-amplification and analysis according to the present method. The Amelogenin locus is identified by GenBank as HUMAMELY (when used to identify a locus on the Y chromosome contained in male DNA) or as HUMAMELX (when used to identify a locus on the X chromosome in male or female DNA). When the Amelogenin locus is co-amplified in the same multiplex amplification reaction as the set of at least thirteen short tandem repeat loci, the sequence of at least one of the primers used to amplify this particular locus in the multiplex amplification reaction preferably has a sequence selected from: SEQ ID NO:86, SEQ ID NO:105, and SEQ ID NO:87.

D. Selection of Primers

Once a set of loci for co-amplification in a single multiplex reaction is identified, one can determine primers suitable for co-amplifying each locus in the set. Care should be used in selecting the sequence of primers used in the multiplex reaction. Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplification at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex. Primers used in the present method or included in the present kits of the invention are preferably selected according to the following selection process.

Primers are preferably developed and selected for use in the multiplex systems of the invention by employing a re-iterative process of selecting primer sequences, mixing the primers for co-amplification of the selected loci, co-amplifying the loci, then separating and detecting the amplified products. Initially, this process often produces the amplified alleles in an imbalanced fashion (i.e., higher product yield for some loci than for others) and may also generate amplification products which do not represent the alleles themselves. These extra fragments may result from any number of causes described above.

To eliminate such extra fragments from the multiplex systems, individual primers from the total set are used with primers from the same or other loci to identify which primers contribute to the amplification of the extra fragments. Once two primers which generate one or more of the fragments are identified, one or both contributors are modified and retested, either in a pair alone or in the multiplex system (or a subset of the multiplex system). This process is repeated until evaluation of the products yields amplified alleles with no or an acceptable level of extra fragments in the multiplex system.

On occasion, extra fragments can be eliminated by labeling the opposite primer in a primer pair. This change reveals the products of the opposing primer in the detection step. This newly labeled primer may amplify the true alleles with greater fidelity than the previously labeled primer generating the true alleles as a greater proportion of the total amplification product.

The determination of primer concentration may be performed either before or after selection of the final primer sequences, but is preferably performed after that selection. Generally, increasing primer concentration for any particular locus increases the amount of product generated for that locus. However, this is also a re-iterative process because increasing yield for one locus may decrease it for one or more other loci. Furthermore, primers may interact directly affecting yield of the other loci. Linear increases in primer concentration do not necessarily produce linear increases in product yield for the corresponding locus.

Locus to locus balance is also affected by a number of parameters of the amplification protocol such as the amount of template used, the number of cycles of amplification, the annealing temperature of the thermal cycling protocol, and the inclusion or exclusion of an extra extension step at the end of the cycling process. Absolutely even balance across all alleles and loci is generally not achieved.

The process of multiplex system development may also be a re-iterative process in another sense described, above. That is, it is possible, first, to develop a multiplex system for a small number of loci, this system being free or nearly free of extra fragments from amplification. Primers of this system may be combined with primers for one or more additional loci. This expanded primer combination may or may not produce extra fragments from amplification. In turn, new primers may be introduced and evaluated.

One or more of the re-iterative selection processes described above are repeated until a complete set of primers is identified which can be used to co-amplify the at least thirteen loci selected for co-amplification as described above. It is understood that many different sets of primers may be developed to amplify a particular set of loci.

Synthesis of the primers used in the present method can be conducted using any standard procedure for oligonucleotide synthesis known to those skilled in the art. At least one primer for each locus is preferably covalently attached to a dye label, as described in Section F, below.

Table 1, below, provides a list sequences of primers which have been determined to be suitable for use in amplifying the corresponding polymorphic STR loci listed therein. At least one primer listed in Table 1 is preferably used to amplify at least one of the loci selected for co-amplification and analysis as described above. It is understood that other primers could be identified which are suitable for simultaneous amplification of the loci listed below.

TABLE 1

Locus	Primer SEQ ID NO:'s
D7S820	1, 2, 80 and 81
D13S317	3, 4, 82 and 83
D5S818	5, 6, 84 and 85
D3S1539	7, 8 and 49
D17S1298	9 and 10
D20S481	11, 12, 52 and 53
D9S930	13, 14, 55 and 61
D10S1239	15, 16 and 54
D14S118	17 and 18
D14S562	19 and 20
D14S548	21 and 22
D16S490	23 and 24
D16S753	25 and 26
D17S1299	27 and 28
D16S539	29, 30, 58, 79 and 97
D22S683	31 and 32
HUMCSF1PO	33, 34, 77, 78 and 98
HUMTPOX	35, 36, 72 and 73
HUMTH01	37, 38, 66, 67 and 103
HUMvWFA31	39, 40, 59, 60 and 76
HUMF13A01	41 and 42
HUMFESFPS	43 and 44
HUMBFXIII	45 and 46

TABLE 1-continued

Locus	Primer SEQ ID NO:'s
HUMLIPOL	47 and 48
D19S253	50 and 51
D4S2368	56 and 57
D18S51	62, 63, 101 and 102
D21S11	64 and 65
D351358	68, 69 and 106
HUMFIBRA	70, 71 and 107
D8S1179	74, 75 and 104
G475	88, 89 and 94
S159	90, 91, 92, 93, 95 and 96
C221	99 and 100

E. Preparation of DNA Samples

Samples of genomic DNA can be prepared for use in the method of this invention using any method of DNA preparation which is compatible with the amplification of DNA. Many such methods are known by those skilled in the art. Examples include, but are not limited to DNA purification by phenol extraction (Sambrook, J., et al. (1989) *Molecular Cloning: A Laboratory Manual, Second Edition,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 9.14–9.19), and partial purification by salt precipitation (Miller, S. et al. (1988) *Nucl. Acids Res.* 16:1215) or chelex (Walsh et al., (1991) *BioTechniques* 10:506–513, Comey, et al., (1994) *Forensic Sci.* 39:1254) and the release of unpurified material using untreated blood (Burckhardt, J. (1994) *PCR Methods and Applications* 3:239–243, McCabe, Edward R. B.,(1991) *PCR Methods and Applications* 1:99–106, Nordvåg, Bjørn-Yngvar (1992) *BioTechniques* 12:4 pp. 490–492).

When the at least one DNA sample to be analyzed using the method of this invention is human genomic DNA, the DNA is preferably prepared from tissue, selected from the group consisting of blood, semen, vaginal cells, hair, saliva, urine, bone, buccal samples, amniotic fluid containing placental cells or fetal cells, chorionic villus, and mixtures of any of the tissues listed above.

Optionally, DNA concentrations can be measured prior to use in the method of the present invention, using any standard method of DNA quantification known to those skilled in the art. In such cases, the DNA concentration is preferably determined by spectrophotometric measurement as described by Sambrook, J., et al. (1989), supra, Appendix E.5, or fluorometrically using a measurement technique such as that described by Brunk C. F., et al. (1979), *Anal Biochem* 92: 497–500. The DNA concentration is more preferably measured by comparison of the amount of hybridization of DNA standards with a human-specific probe such as that described by Waye, J. S., et al. (1991) "Sensitive and specific quantification of human genomic deoxyribonucleic acid (DNA) in forensic science specimens: casework examples," *J. Forensic Sci.,* 36:1198–1203. Use of too much template DNA in the amplification reactions can produce artifacts which appear as extra bands which do not represent true alleles.

F. Amplification of DNA

Once a sample of genomic DNA is prepared, the targeted loci can be co-amplified in the multiplex amplification step of the present method. Any one of a number of different amplification methods can be used to amplify the loci, including, but not limited to, polymerase chain reaction (PCR) (Saiki, R. K., et al. (1985), *Science* 230: 1350–1354), transcription based amplification (Kwoh, D. Y., and Kwoh, T. J. (1990), *American Biotechnology Laboratory,* October, 1990) and strand displacement amplification (SDA) (Walker, G. T., et al. (1992) *Proc. Natl. Acad. Sci., U.S.A.* 89: 392–396). Preferably, the DNA sample is subjected to PCR amplification using primer pairs specific to each locus in the set. Reference is made to the Sequence Listing at the end of this specification for details of the primer sequences used in the Examples below, some of which sequences are alternative embodiments of this invention.

At least one primer for each locus is preferably covalently attached to a dye label, more preferably a fluorescent dye label. The primers and dyes attached thereto are preferably selected for the multiplex amplification reaction, such that alleles amplified using primers for each locus labeled with one color do not overlap the alleles of the other loci in the set co-amplified therein using primers labeled with the same color, when the alleles are separated, preferably, by gel or capillary electrophoresis.

In a particularly preferred embodiment of the method of the present invention, at least one primer for each locus co-amplified in the multiplex reaction is labeled with a fluorescent label prior to use in the reaction. Fluorescent labels suitable for attachment to primers for use in the present invention are commercially available. See, e.g. fluorescein and carboxy-tetramethylrhodamine labels and their chemical derivatives from PE Biosystems and Molecular Probes. Most preferably, at least three different labels are used to label the different primers used in the multiplex amplification reaction. When a size marker is included to evaluate the multiplex reaction, the primers used to prepare the size marker are preferably labeled with a different label from the primers used to amplify the loci of interest in the reaction.

Details of the most preferred amplification protocol for each of the most preferred combinations of loci for use in the method of this invention are given in the Examples below. Reference is also made to the Examples for additional details of the specific procedure relating to each multiplex. The sequences of the locus-specific primers used in the Examples include a number of nucleotides which, under the conditions used in the hybridization, are sufficient to hybridize with an allele of the locus to be amplified and to be essentially free from amplification of alleles of other loci. Reference is made to U.S. Pat. No. 5,192,659 to Simons, the teaching of which is incorporated herein by reference for a more detailed description of locus-specific primers.

G. Separation and Detection of DNA Fragments

Once a set of amplified alleles is produced from the multiplex amplification step of the present method, the amplified alleles are evaluated. The evaluation step of this method can be accomplished by any one of a number of different means, the most preferred of which are described below.

Electrophoresis is preferably used to separate the products of the multiplex amplification reaction, more preferably capillary electrophoresis (see, e.g., Buel, Eric et al. (1998), *Journal of Forensic Sciences;* 43:(1) pp. 164–170) or denaturing polyacrylamide gel electrophoresis (see, e.g., Sambrook, J. et al. (1989) *In Molecular Cloning—A Laboratory Manual,* 2nd edition, Cold Spring Harbor Laboratory Press, pp. 13.45–13.57). Gel preparation and electrophoresis procedures and conditions for suitable for use in the evaluating step of the method of this invention are illustrated in the Examples, below. Separation of DNA fragments in a denaturing polyacrylamide gel and in capillary electrophoresis occurs based primarily on fragment size.

Once the amplified alleles are separated, the alleles and any other DNA in the gel or capillary (e.g., DNA size markers or an allelic ladder) can then be visualized and

analyzed. Visualization of the DNA in the gel can be accomplished using any one of a number of prior art techniques, including silver staining or reporters such as radioisotopes, fluorescers, chemiluminescers and enzymes in combination with detectable substrates. However, the preferred method for detection of multiplexes containing thirteen or more loci is fluorescence (see, e.g., Schumm, J. W. et al. in *Proceedings from the Eighth International Symposium on Human Identification,* (pub. 1998 by Promega Corporation), pp. 78–84; Buel, Eric et al. (1998), supra.), wherein primers for each locus in the multiplexing reaction is followed by detection of the labeled products employing a fluorometric detector. The references cited above, which describe prior art methods of visualizing alleles, are incorporated by reference herein.

The alleles present in the DNA sample are preferably determined by comparison to a size standard such as a DNA marker or a locus-specific allelic ladder to determine the alleles present at each locus within the sample. The most preferred size marker for evaluation of a multiplex amplification containing two or more polymorphic STR loci consists of a combination of allelic ladders for each of the loci being evaluated. See, e.g., Puers, Christoph et al., (1993) *Am J. Hum Genet.* 53:953–958, Puers, Christoph, et al. (1994) *Genomics* 23:260–264. See also, U.S. Pat. Nos. 5,599,666; 5,674,686; and 5,783,406 for descriptions of allelic ladders suitable for use in the detection of STR loci, and methods of ladder construction disclosed therein.

Following the construction of allelic ladders for individual loci, these may be mixed and loaded for gel electrophoresis at the same time as the loading of amplified samples occurs. Each allelic ladder co-migrates with alleles in the sample from the corresponding locus.

The products of the multiplex reactions of the present invention can be evaluated using an internal lane standard, a specialized type of size marker configured to run in the same lane of a polyacrylamide gel or same capillary. The internal lane standard preferably consists of a series of fragments of known length. The internal lane standard more preferably is labeled with a fluorescent dye which is distinguishable from other dyes in the amplification reaction.

Following construction of the internal lane standard, this standard can also be mixed with amplified sample or allelic ladders and loaded for electrophoresis for comparison of migration in different lanes of gel electrophoresis or different capillaries of capillary electrophoresis. Variation in the migration of the internal lane standard indicates variation in the performance of the separation medium. Quantitation of this difference and correlation with the allelic ladders allows correction in the size determination of alleles in unknown samples.

H. Preferred Detection Technique: Fluorescent Detection

In one of the most preferred embodiments of the method of this invention, fluorescent detection is used to evaluate the amplified alleles in the mixture produced by the multiplex amplification reaction. Below is a brief summary of how that method of detection preferably is practiced.

With the advent of automated fluorescent imaging, faster detection and analysis of multiplex amplification products can be achieved. For fluorescent analysis, one fluorescent labeled primer can be included in the amplification of each locus. Fluorescent labeled primers preferably suited for use in the present invention include the fluorescein-labeled (FL-), carboxy-tetramethylrhodamine-labeled (TMR-), and 5,6-carboxyrhodamine 6G-labeled (R6G) primers, such as are illustrated in the Examples, below. Separation of the amplified fragments produced using such labeled primers is achieved preferably by slab gel electrophoresis or capillary electrophoresis. The resulting separated fragments can be analyzed using fluorescence detection equipment such as an ABI PRISM® 310 Genetic Analyzer, an ABI PRISM® 377 DNA Sequencer (Applied Biosystems Division, Perkin Elmer, Foster City, Calif.), or a Hitachi FMBIO® II Fluorescent Scanner (Hitachi Software Engineering America, Ltd. South San Francisco, Calif.).

In summary, the method of this invention is most preferably practiced using fluorescent detection as the detection step. In this preferred method of detection, one or both of each pair of primers used in the multiplex amplification reaction has a fluorescent label attached thereto, and as a result, the amplified alleles produced from the amplification reaction are fluorescently labeled. In this most preferred embodiment of the invention, the amplified alleles are subsequently separated by capillary electrophoresis and the separated alleles visualized and analyzed using a fluorescent image analyzer.

Fluorescent detection is preferred over radioactive methods of labeling and detection, because it does not require the use of radioactive materials, and all the regulatory and safety problems which accompany the use of such materials.

Fluorescent detection employing labeled primers is also preferred over other non-radioactive methods of detection, such as silver staining, because fluorescent methods of detection generally reveal fewer amplification artifacts than silver staining. The smaller number of artifacts are due, in part, to the fact that only amplified strands of DNA with labels attached are detected in fluorescent detection, while both strands of every amplified allele of DNA produced from the multiplex amplification reaction is stained and detected using the silver staining method of detection.

I. Kit

The present invention is also directed to kits that utilize the process described above. A basic kit comprises a container having one or more locus-specific primers. Instructions for use optionally may be included.

Other optional kit components may include an allelic ladder directed to each of the specified loci, a sufficient quantity of enzyme for amplification, amplification buffer to facilitate the amplification, loading solution for preparation of the amplified material for electrophoresis, genomic DNA as a template control, a size marker to insure that materials migrate as anticipated in the separation medium, and a protocol and manual to educate the user and to limit error in use. The amounts of the various reagents in the kits also can be varied depending upon a number of factors, such as the optimum sensitivity of the process. It is within the scope of this invention to provide test kits for use in manual applications or test kits for use with automated detectors or analyzers.

EXAMPLES

The following Examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. The Examples are intended to be illustrative, and are not intended in any way to otherwise limit the scope of the claims or protection granted by the patent.

The human genomic DNA samples assayed in the Example below were prepared from blood or tissue culture cells, using a standard procedure described by Miller and Dykes in (Miller, S. et al. (1988) *Nucl. Acids Res.* 16:1215). The isolation and quantification methods described therein are generally known to those skilled in the art and are preferred, but not required, for application of the invention.

Each Example below is an example of the use of the method of this invention, to determine simultaneously the alleles present in at least thirteen loci from one or more DNA samples of human genomic DNA. Each set of loci co-amplified below includes the thirteen short tandem repeat loci identified for use in the CODIS system (i.e., D3S1358, HUMTH01, D21S11, D18S51, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D13S317, D7S820, D16S539, and HUMCSF1PO). Some sets of loci co-amplified below also include one or more additional short tandem repeat loci, such as loci with pentanucleotide repeats (e.g., G475, S159, or C221), and a non-STR locus, Amelogenin.

Table 2 summarizes which set of loci was co-amplified in the multiplex amplification reaction described in each Example below. The table also indicates which primer pair was used to amplify each such locus in each such multiplex reaction. One primer of each primer pair listed on Table 2 was fluorescently labeled prior to being used in the multiplex amplification reaction. In some cases, a different label was used to label primers to different loci, such that the alleles produced using the different primers could be distinguished from one another when detected with a laser-activated fluorescence detection device.

Three different fluorescent labels were used in the Examples below, described as "FL" to indicate fluorescein-labeled, "TMR" to indicate carboxy-tetramethylrhodamine-labeled, and "R6G" to indicate 5,6-carboxyrhodamine 6G in Table 2, below. Table 2 also indicates which primer of each pair of primers used in the multiplex amplification reaction was so labeled in each Example (e.g., "FL-69" means the primer with SEQ ID NO:69 was labeled at its 5' end with fluorescein prior to being used in the multiplex amplification reaction). In the text of each of the Examples, however, the label abbreviation is placed immediately before the SEQ ID NO of the labeled primer used in the amplification reaction described therein (e.g., "FL-SEQ ID NO:2" instead of "FL-2").

TABLE 2

Example	Loci Amplified	Primer Pair: SEQ ID NO's Used	Fluorescent Label(s) Used
1	D3S1358	68,69	FL-69
	HUMTHO1	66,67	FL-66
	D21S11	64,65	FL-65
	D18S51	62,63	FL-62
	HUMvWFA31	76,40	TMR-40
	D8S1179	74,75	TMR-75
	HUMTPOX	72,73	TMR-73
	HUMFIBRA	70,71	TMR-70
	D5S818	84,85	R6G-85
	D13S317	82,83	R6G-83
	D7S820	80,81	R6G-80
	D16S539	29,79	R6G-79
	HUMCSF1PO	77,78	R6G-78
2,3	D3S1358	68,69	FL-69
	HUMTHO1	66,67	FL-66
	D21S11	64,65	FL-65
	D18S51	62,63	FL-62
	G475	88,89	FL-88
	Amelogenin	86,87	TMR-86
	HUMvWFA31	76,40	TMR-40
	D8S1179	74,75	TMR-75
	HUMTPOX	72,73	TMR-73
	HUMFIBRA	70,71	TMR-70
	D5S818	84,85	R6G-85
	D13S317	82,83	R6G-83
	D7S820	80,81	R6G-80
	D16S539	29,79	R6G-79
	HUMCSF1PO	77,78	R6G-78
4	D3S1358	68,69	FL-69
	HUMTHO1	66,67	FL-66
	D21S11	64,65	FL-65
	D18S51	62,63	FL-62
	G475	88,89	FL-88
	Amelogenin	86,87	TMR-86
	HUMvWFA31	76,40	TMR-40
	D8S1179	74,75	TMR-75
	HUMTPOX	72,73	TMR-73
	HUMFIBRA	70,71	TMR-70
	D5S818	84,85	FL-85
	D13S317	82,83	FL-83
	D7S820	80,81	FL-80
	D16S539	29,79	FL-79
	HUMCSF1PO	77,78	FL-78
	S159	90,91	FL-91
5	D3S1358	68,69	FL-69
	HUMTHO1	66,67	FL-66
	D21S11	64,65	FL-65
	D18S51	62,63	FL-62
	G475	88,94	FL-94
	Amelogenin	86,87	TMR-86
	HUMvWFA31	76,40	TMR-40
	D8S1179	74,75	TMR-75
	HUMTPOX	72,73	TMR-73
	HUMFIBRA	70,71	TMR-70
	D5S818	84,85	FL-85
	D13S317	82,83	FL-83
	D7S820	80,81	FL-80
	D16S539	29,79	FL-79
	HUMCSF1PO	77,78	FL-78
	S159	95,96	FL-96
6	D3S1358	69,106	FL-69
	HUMTHO1	38,103	FL-38
	D21S11	64,65	FL-65
	D18S51	101,102	FL-101
	S159	92,93	FL-93
	Amelogenin	105,87	TMR-105
	HUMvWFA31	76,40	TMR-40
	D8S1179	104,75	TMR-104
	HUMTPOX	72,73	TMR-72
	HUMFIBRA	70,107	TMR-70
	D5S818	84,85	FL-85
	D13S317	3,4	FL-4
	D7S820	80,81	FL-80
	D16S539	29,97	FL-29
	HUMCSF1PO	77,98	FL-98
	C221	99,100	FL-99

Example 1

Fluorescent Detection of Multiplex Amplification of Loci D3S1358. HUMTH01, D21S11, D18S51, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, and HUMCSF1PO as Detected with the ABI PRISM® 310 Genetic Analyzer

In this Example, a DNA template was amplified simultaneously at the individual loci D3S1358, HUMTH01, D21S11, D18S51, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, and HUMCSF1PO in a single reaction vessel. The PCR amplification was performed in 25 μl of 1×Gold ST*R Buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 160 μg/ml BSA and 200 μM each of dATP, dCTP, dGTP and dTTP) using 1 ng template, and 3.25 U AmpliTaq Gold™ DNA Polymerase. A GeneAmp® PCR System 9600 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C.

for 12 min., then 10 cycles of 94° C. for 30 sec., ramp for 68 sec. to 58° C., hold for 30 sec., ramp 50 sec. to 70° C., hold for 45 sec., followed by 20 cycles of 90° C. for 30 sec., ramp 60 sec. to 58° C., hold for 30 sec., ramp for 50 sec, to 70° C., hold for 45 sec., followed by 1 cycle of 60° C. for 30 min.

Twenty-six amplification primers were used in combination, including 0.12 μM each D3S1358 primers 1 [SEQ ID NO:68] and 2 [FL-SEQ ID NO:69], 0.08 μM each HUMTH01 primers 1 [FL-SEQ ID NO:66] and 2 [SEQ ID NO:67], 0.3 μM each D21S11 primers 1 [SEQ ID NO:64] and 2 [FL-SEQ ID NO:65], 0.2 μM each D18S51 primers 1 [FL-SEG ID NO:62] and 2 [SEQ ID NO:63], 1.1 μM each HUMvWFA31 primers 1 [SEQ ID NO:76] and 2 [TMR-SEQ ID NO:40], 1.8 μM each D8S1179 primers 1 [SEQ ID NO:74] and 2 [TMR-SEQ ID NO:75], 0.6 μM each HUMTPOX primers 1 [SEQ ID NO:72] and 2 [TMR-SEQ ID NO:73], 2.4 μM each HUMFIBRA primers 1 [TMR-SEQ ID NO:70] and 2 [SEQ ID NO:71], 0.2 μM each D5S818 primers 1 [SEQ ID NO:84] and 2 [R6G-SEQ ID NO:85], 0.1 μM each D13S317 primers 1 [SEQ ID NO:82] and 2 [R6G-SEQ ID NO:83], 0.2 μM each D7S820 primers 1 [R6G-SEQ ID NO:80]and 2 [SEQ ID NO:81], 0.15 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [R6G-SEQ ID NO:79], 0.2 μM each HUMCSF1PO primers 1 [SEQ ID NO:77] and 2 [R6G-SEQ ID NO:78]

Amplified products were separated using an ABI PRISM® 310 Genetic Analyzer. DNA samples were mixed with 24 μl of a loading solution (deionized formamide) and 1.0 μl of an internal lane size standard, denatured at 95° C. for 3 min., and chilled on ice prior to injection. Separation was carried out using Performance Optimized Polymer 4 (POP-4)(Perkin Elmer Biosystems, Foster City, Calif.) in a 47 cm×50 μm capillary. The manufacturer's GeneScan® run module GS STR POP4 (Id.) (1 ml) A was used. Conditions for the electrophoresis were a 5 second injection, injection kV was 15.0, run kV was 15.0, run temperature was 60° C., run time was 28 minutes and virtual filter A was used.

FIG. **1**A is a printout of results of scanning the amplified fragments of each locus separated and detected with the ABI PRISM® 310 Genetic Analyzer, as described above. FIG. **1**A shows amplification products of a DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, and HUMCSF1PO. Peaks shown in Panel A are labeled with fluorescein, peaks shown in Panel B are labeled with carboxy-tetramethylrhodamine, and peaks shown in Panel C are labeled with 5,6carboxyrhodamine 6G.

FIG. **1**B is a printout of the results of scanning a sample prepared in the same way as the sample scanned in FIG. **1**A, except that no DNA template was used in the amplification reaction. Peaks in this figure are background products resulting from dye conjugation and purification procedures and from undefined causes.

Example 2

Fluorescent Detection of Multiplex Amplification of Loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159 as detected with the ABI PRISM® 310 Genetic Analyzer

In this Example, a DNA template was amplified simultaneously at the individual loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159 in a single reaction vessel. The PCR amplification was performed in 25 μl of 1×Gold ST*R Buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 160 μg/ml BSA and 200 μM each of dATP, dCTP, dGTP and dTTP) using 1 ng template, and 4 U AmpliTaq Gold™ DNA Polymerase. A GeneAmp® PCR System 9600 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 12 min., then 10 cycles of 94° C. for 30 sec., ramp for 68 sec. to 58° C., hold for 30 sec., ramp 50 sec. to 70° C., hold for 45 sec., followed by 20 cycles of 90° C. for 30 sec., ramp 60 sec. to 58° C., hold for 30 sec., ramp for 50 sec, to 70° C., hold for 45 sec., followed by 1 cycle of 60° C. for 30 min.

Thirty-two amplification primers were used in combination, including 0.12 μM each D3S1358 primers 1 [SEQ ID NO:68] and 2 [FL-SEQ ID NO:69], 0.08 μM each HUMTH01 primers 1 [FL-SEQ ID NO:66] and 2 [SEQ ID NO:67], 0.3 μM each D21S11 primers 1 [SEQ ID NO:64] and 2 [FL-SEQ ID NO:65], 0.2 μM each D18S51 primers 1 [FL-SEQ ID NO:62] and 2 [SEQ ID NO:63], 0.24 μM each G475 primers 1 [FL-SEQ ID NO:88] and 2 [SEQ ID NO:89], 0.6 μM each Amelogenin primers 1 [TMR-SEQ ID NO:86] and 2 [SEQ ID NO:87], 1.1 μM each HUMvWFA31 primers 1 [SEQ ID NO:76] and 2 [TMR-SEQ ID NO:40], 1.8 μM each D8S1179 primers 1 [SEQ ID NO:74] and 2 [TMR-SEQ ID NO:75], 0.6 μM each HUMTPOX primers 1 [SEQ ID NO:72] and 2 [TMR-SEQ ID NO:73], 2.4 μM each HUMFIBRA primers 1 [TMR-SEQ ID NO:70] and 2 [SEQ ID NO:71], 0.2 μM each D5S818 primers 1 [SEQ ID NO:84] and 2 [R6G-SEQ ID NO:85], 0.1 μM each D13S317 primers 1 [SEQ ID NO:82] and 2 [R6G-SEQ ID NO:83], 0.2 μM each D7S820 primers 1 [R6G-SEQ ID NO:80] and 2 [SEQ ID NO:81], 0.15 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [R6G-SEQ ID NO:79], 0.2 μM each HUMCSF1PO primers 1 [SEQ ID NO:77] and 2 [R6G-SEQ ID NO:78] 0.1 μM each S159 primers 1 [SEQ ID NO:90] and 2 [R6G-SEQ ID NO:91].

Amplified products were separated using an ABI PRISM® 310 Genetic Analyzer. DNA samples were mixed with 24 μl of a loading solution (deionized formamide) and 1.0 μl of an internal lane size standard, denatured at 95° C. for 3 min., and chilled on ice prior to injection. Separation was carried out using Performance Optimized Polymer 4 (POP-4) (Perkin Elmer Biosystems, Foster City, Calif.) in a 47 cm×50 μm capillary. The manufacturer's GeneScan® run module GS STR POP4 (Id.)(1 ml) A was used. Conditions for the electrophoresis were a 5 second injection, injection kV was 15.0, run kV was 15.0, run temperature was 60° C., run time was 28 minutes and virtual filter A was used.

FIG. **2**A is a printout of results of scanning the amplified fragments of each locus separated and detected with the ABI PRISM® 310 Genetic Analyzer, as described above. FIG. **2**A shows amplification products of a DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO, and S159. Peaks shown in Panel A are labeled with fluorescein, peaks shown in Panel B are labeled with carboxy-tetramethylrhodamine, and peaks shown in Panel C are labeled with 5,6carboxyrhodamine 6G.

FIG. **2**B is a printout of the results of scanning a sample prepared in the same way as the sample scanned in FIG. **2**A, except that no DNA template was used in the amplification

reaction. Peaks in this figure are background products resulting from dye conjugation and purification procedures and from undefined causes.

Example 3

Fluorescent Detection of Multiplex Amplification of Loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO, and S159 as detected with the ABI PRISM® 377 DNA Sequencer

In this Example, a DNA template was amplified as in Example 2. Amplified products were separated using an ABI PRISM® 377 DNA Sequencer. This was carried out using a 0.2 mm thick, 5% Long Ranger™ Acrylamide (FMC BioProducts, Rockland, Me.), 7M urea gel. DNA samples were mixed with 1.5 μl of a loading solution (88.25% formamide, 4.1 mM EDTA, 15 mg/ml Blue Dextran) and 0.5 μl of an internal lane size standard, denatured at 95° C. for 2 min., and chilled on ice prior to loading. Electrophoresis was carried out using the manufacturer's GeneScan® modules for Prerun (PR GS 36A-2400) and Run (GS 36A-2400). Run time was 3 hours and virtual filter A was used.

FIG. **3**A is a printout of results of scanning the amplified fragments of each locus separated and detected with the ABI PRISM® 377 DNA Sequencer, as described above. FIG. **3**A shows amplification products of a DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO, and S159. Peaks shown in Panel A are labeled with fluorescein, peaks shown in Panel B are labeled with carboxy-tetramethylrhodamine, and peaks shown in Panel C are labeled with 5,6carboxyrhodamine 6G.

FIG. **3**B is a printout of the results of scanning a sample prepared in the same way as the sample scanned in FIG. **3**A, except that no DNA template was used in the amplification reaction. Peaks in this figure are background products resulting from dye conjugation and purification procedures and from undefined causes.

Example 4

Fluorescent Detection of Multiplex Amplification of Loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO, and S159 as Detected with the Hitachi FMBIO® II Fluorescent Scanner

In this example, two DNA templates were each amplified simultaneously at each of three different locus combinations selected from the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159. Amplification of each locus combination included 5 ng template in a single reaction vessel containing 25 μl of 1×Gold ST*R Buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 160 μg/ml BSA and 200 μM each of dATP, dCTP, dGTP and dTTP).

A GeneAmp® PCR System 9600 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 12 min., then 10 cycles of 94° C. for 30 sec., ramp for 68 sec. to 58° C., hold for 30 sec., ramp 50 sec. to 70° C., hold for 45 sec., followed by 22 cycles of 90° C. for 30 sec., ramp 60 sec. to 58° C., hold for 30 sec., ramp for 50 sec, to 70° C., hold for 45 sec., followed by 1 cycle of 60° C. for 30 min.

Thirty-two amplification primers were used in the following concentrations, including 0.225 μM each D3S1358 primers 1 [SEQ ID NO:68] and 2 [FL-SEQ ID NO:69], 0.2 μM each HUMTH01 primers 1 [FL-SEQ ID NO:66] and 2 [SEQ ID NO:67], 1.0 μM each D21S11 primers 1 [SEQ ID NO:64] and 2 [FL-SEQ ID NO:65], 1.0 μM each D18S51 primers 1 [FL-SEQ ID NO:62] and 2 [SEQ ID NO:63], 2.8 μM each G475 primers 1 [FL-SEQ ID NO:88] and 2 [SEQ ID NO:89], 0.2 μM each Amelogenin primers 1 [TMR-SEQ ID NO:86] and 2 [SEQ ID NO:87], 0.3 μM each HUMvWFA31 primers 1 [SEQ ID NO:76] and 2 [TMR-SEQ ID NO:40], 1,5 μM each D8S1179 primers 1 [SEQ ID NO:74] and 2 [TMR-SEQ ID NO:75], 0.2 μM each HUMTPOX primers 1 [SEQ ID NO:72] and 2 [TMR-SEQ ID NO:73], 2.0 μM each HUMFIBRA primers 1 [TMR-SEQ ID NO:70] and 2 [SEQ ID NO:71], 0.55 μM each D5S818 primers 1 [SEQ ID NO:84] and 2 [FL-SEQ ID NO:85], 1.1 μM each D13S317 primers 1 [SEQ ID NO:82] and 2 [FL-SEQ ID NO:83], 1.7 μM each D7S820 primers 1 [FL-SEQ ID NO:80] and 2 [SEQ ID NO:81], 3.3 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:79], 0.5 μM each HUMCSF1PO primers 1 [SEQ ID NO:77] and 2 [FL-SEQ ID NO:78], 2.0 μM each S159 primers 1 [SEQ ID NO:90] and 2 [FL-SEQ ID NO:91].

In the first locus combination, each template was amplified using 2.5 U of AmpliTaq Gold™ DNA Polymerase and primers for each locus used in the concentrations described above for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA. In the second locus combination, all thirty-two primers, above, at the described concentrations, and 4 U of AmpliTaq Gold™ DNA Polymerase were used to amplify DNA templates at all sixteen loci, D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159 in a single reaction vessel. In the third combination, each template was amplified using 1.5 U of AmpliTaq Gold™ DNA Polymerase and primers for each locus used in the concentrations described above for the loci D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159.

Amplification products were separated by electrophoresis through a 0.4 mm thick 4% denaturing polyacrylamide gel (19:1 ratio of acrylamide to bis-acrylamide) which contained 7 M urea (Sambrook et al., (1989)), and which was chemically cross-linked to 2 glass plates (Kobayashi, Y. (1988), *BRL Focus* 10: 73–74). DNA samples were mixed with 3.5 μl of a loading solution (10 mM NaOH, 95% formamide, 0.05% bromophenol blue) and 0.5 μl of an internal lane size standard, denatured at 95° C. for 2 min., and chilled on ice prior to loading. The separated products were visualized by detection of the fluorescent signals using the Hitachi FMIBO® II fluorescent scanner (Hitachi Software Engineering America, Ltd. South San Francisco, Calif.). Band pass filters at 505 nm and 585 nm, respectively, were used for the detection of fluorescein-labeled loci and carboxy-tetramethylrhodamine-labeled loci, respectively. A band pass filter of 650 nm was used for detection of the internal lane standard (size standard data, not shown).

Reference is made to FIGS. **4**A and **4**B, which display the fragments resulting from each amplification reaction. FIG.

4A shows the results from the 505 nm scan (Fluorescein channel) and FIG. 4B shows the results from the 585 nm scan (carboxy-tetramethylrhodamine channel) of the same lanes of the polyacrylamide gel. For each DNA template, lane 1 shows the results of the DNA sample which has been simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA. Lane 2 shows the results of the DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, and S159. Lane 3 shows the results of the DNA sample simultaneously co-amplified for the loci D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, and S159.

Example 5

Fluorescent Detection of Multiplex Amplification of Loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO, and S159 as detected with the Hitachi FMBIO® II Fluorescent Scanner

In this example, two DNA templates were each amplified simultaneously at each of two different locus combinations selected from the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159. Amplification of each locus combination included 5 ng template in a single reaction vessel containing 25 μl of 1×Gold ST*R Buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 160 μg/ml BSA and 200 μM each of dATP, dCTP, dGTP and dTTP).

A GeneAmp® PCR System 9600 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 12 min., then 10 cycles of 94° C. for 30 sec., ramp for 68 sec. to 58° C., hold for 30 sec., ramp 50 sec. to 70° C., hold for 45 sec., followed by 22 cycles of 90° C. for 30 sec., ramp 60 sec. to 58° C., hold for 30 sec., ramp for 50 sec, to 70° C., hold for 45 sec., followed by 1 cycle of 60° C. for 30 min.

Thirty-two amplification primers were used in the following concentrations, including 0.225 μM each D3S1358 primers 1 [SEQ ID NO:68] and 2 [FL-SEQ ID NO:69], 0.2 μM each HUMTH01 primers 1 [FL-SEQ ID NO;66] and 2 [SEQ ID NO:67], 1.0 μM each D21S11 primers 1 [SEQ ID NO:64] and 2 [FL-SEQ ID NO:65], 1.0 M each D18S51 primers 1 [FL-SEQ ID NO:62] and 2 [SEQ ID NO:63], 2.8 μM each G475 primers 1 [SEQ ID NO:88] and 2 [FL-SEQ ID NO:94], 0.2 μM each Amelogenin primers 1 [TMR-SEQ ID NO:86] and 2 [SEQ ID NO:87], 0.3 μM each HUMvWFA31 primers 1 [SEQ ID NO:76] and 2 [TMR-SEQ ID NO:40], 1.5 μM each D8S1179 primers 1 [SEQ ID NO:74] and 2 [TMR-SEQ ID NO:75], 0.2 μM each HUMTPOX primers 1 [SEQ ID NO:72] and 2 [TMR-SEQ ID NO:73], 2.0 μM each HUMFIBRA primers 1 [TMR-SEQ ID NO:70] and 2 [SEQ ID NO:71], 0.55 μM each D5S818 primers 1 [SEQ ID NO:84] and 2 [FL-SEQ ID NO:85], 1.1 μM each D13S317 primers 1 [SEQ ID NO:82] and 2 [FL-SEQ ID NO:83], 1.7 μM each D7S820 primers 1 [FL-SEQ ID NO:80] and 2 [SEQ ID NO:81], 3.3 μM each D16S539 primers 1 [SEQ ID NO:29] and 2 [FL-SEQ ID NO:79], 0.5 μM primers 1 [TMR-SEQ ID NO:104] and 2 [SEQ ID NO:75], 0.75 μM each HUMTPOX primers 1 [TMR-SEQ ID NO:72] and 2 [SEQ ID NO:73], 1.5 μM each HUMFIBRA primers 1 [TMR-SEQ ID NO:70] and 2 [SEQ ID NO:107], 0.55 μM each D5S818 primers 1 [SEQ ID NO:84] and 2 [FL-SEQ ID NO:85], 1.1 μM each D13S317 primers 1 [SEQ ID NO:3] and 2 [FL-SEQ ID NO-4], 1.7 μM each D7S820 primers 1 [FL-SEQ ID NO:80] and 2 [SEQ ID NO:81], 3.3 μM each D16S539 primers 1 [FL-SEQ ID NO:29] and 2 [SEQ ID NO:97], 0.25 μM each HUMCSF1PO primers 1 [SEQ ID NO:77] and 2 [FL-SEQ ID NO:98], 1.0 μM each C221 primers 1 [FL-SEQ ID NO:99] and 2 [SEQ ID NO:100].

In the first locus combination, each template was amplified using 2.5 U of AmpliTaq Gold™ DNA Polymerase and primers for each locus used in the concentrations described above for the loci D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA. In the second locus combination, all thirty-two primers, above, at the described concentrations and 4 U of AmpliTaq Gold™ DNA Polymerase were used to amplify DNA templates at all sixteen loci, D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and C221 in a single reaction vessel. In the third combination, each template was amplified using 1.5 U of AmpliTaq Gold™ DNA Polymerase and primers for each locus used in the concentrations described above for the loci D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and C221.

The amplification products were separated and detected as described in Example 4, except that each sample of amplification products was diluted 1:4 in 1×STR Buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$, and 200 μM each of dATP, dCTP, dGTP and dTTP). The diluted amplification products (2.5 μl) were mixed with 2.5 μl of a loading solution (10 mM NaOH, 95% formamide, 0.05% bromophenol blue), without an internal lane standard, denatured at 95° C. for 2 min., and chilled on ice prior to loading.

Example 6

Fluorescent Detection of Multiplex Amplification of Loci D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D 13S317, D16S539, HUMCSF1PO, and C221 as Detected with the Hitachi FMBIO® II Fluorescent Scanner

In this example, two DNA templates were each amplified simultaneously at each of three different locus combinations selected from the loci D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and C221. Amplification of each locus combination included 10 ng template in a single reaction vessel containing 25 μl of 1×Gold ST*R Buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3 at 25° C.), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 160 μg/ml BSA and 200 μM each of dATP, dCTP, dGTP and dTTP).

A GeneAmp® PCR System 9600 (Perkin Elmer, Foster City, Calif.) was employed with the following amplification protocol: 96° C. for 12 min., then 10 cycles of 94° C. for 30 sec., ramp for 68 sec. to 60° C., hold for 30 sec., ramp 50 sec. to 70° C., hold for 45 sec., followed by 20 cycles of 90° C. for 30 sec., ramp 60 sec. to 60° C., hold for 30 sec., ramp for 50 sec, to 70° C., hold for 45 sec., followed by 1 cycle of 60° C. for 30 min.

Thirty-two amplification primers were used in the following concentrations, including 0.75 μM each D3S1358 primers 1 [SEQ ID NO:106] and 2 [FL-SEQ ID NO:69], 0.3 μM each HUMTH01 primers 1 [FL-SEQ ID NO:38] and 2 [SEQ ID NO:103], 2.0 μM each D21S11 primers 1 [SEQ ID NO:64] and 2 [FL-SEQ ID NO:65], 0.3 μM each D18S51 primers 1 [FL-SEQ ID NO:101] and 2 [SEQ ID NO:102], 2.0 μM each S159 primers 1 [SEQ ID NO:92] and 2 [FL-SEQ ID NO:93], 0.15 μM each Amelogenin primers 1 [TMR-SEQ ID NO:105] and 2 [SEQ ID NO:87], 1.0 μM each HUMvWFA31 primers 1 [SEQ ID NO:76] and 2 [TMR-SEQ ID NO:40], 1.25 μM each D8S1179 each HUMCSF1PO primers 1 [SEQ ID NO:77] and 2 [FL-SEQ ID NO:78], 2.0 μM each S159 primers 1 [SEQ ID NO:95] and 2 [FL-SEQ ID NO:96].

In the first locus combination, each template was amplified using 2.5 U of AmpliTaq Gold™ DNA Polymerase and primers for each locus used in the concentrations described above for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA. In the second locus combination, all thirty-two primers, above, at the described concentrations, and 4 U of AmpliTaq Gold™ DNA Polymerase were used to amplify DNA templates at all sixteen loci, D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159 in a single reaction vessel.

The separation and visualization of amplified products were as described in Example 4.

Reference is made to FIG. **5**A and **5**B, which display the fragments resulting from each amplification reaction. FIG. **5**A shows the results from the 505 nm scan (Fluorescein channel) and FIG. **5**B shows the results from the 585 nm scan (carboxy-tetramethylrhodamine channel) of the same lanes of the polyacrylamide gel. For each template, lane 1 shows the results of the DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA and lane 2 shows the results of the DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, and S159.

Reference is made to FIGS. **6**A and **6**B, which display the fragments resulting each amplification reaction. FIG. **6**A shows the results from the 505 nm scan (Fluorescein channel) and FIG. **6**B shows the results from the 585 nm scan (carboxy-tetramethylrhodamine channel) of the same lanes of the polyacrylamide gel. For each DNA template, lane 1 shows the results of the DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA. Lane 2 shows the results of the DNA sample simultaneously co-amplified for the loci D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, and C221. Lane 3 shows the results of the DNA sample simultaneously co-amplified for the loci D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, and C221.

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<222> LOCATION: D14S118

<400> SEQUENCE: 17

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<210> SEQ ID NO 18
<211> LENGTH: 24
<212> TYPE: DNA

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<213> ORGANISM: Homo sapien
<222> LOCATION: D14S118

<400> SEQUENCE: 18

caaactcctg aggtcaaaca atcc 24

<210> SEQ ID NO 19
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<212> TYPE: DNA
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<222> LOCATION: D14S562

<400> SEQUENCE: 19

cttggagggt ggggtggcta a 21

<210> SEQ ID NO 20
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D14S562

<400> SEQUENCE: 20

cgaaattttg ttgccttgct ctgg 24

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<222> LOCATION: D14S548

<400> SEQUENCE: 21

cctgggcaac agagtgagac t 21

<210> SEQ ID NO 22
<211> LENGTH: 24
<212> TYPE: DNA
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<400> SEQUENCE: 22

acccagcttt aacagtttgt gctt 24

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<222> LOCATION: D16S490

<400> SEQUENCE: 23

gggcggacac agaatgtaaa atc 23

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<211> LENGTH: 24
<212> TYPE: DNA
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<222> LOCATION: D16S490

<400> SEQUENCE: 24

aaacccaaat agatgacagg caca 24

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<212> TYPE: DNA
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<400> SEQUENCE: 25

gcactccagg ctgaatgaca gaac 24

<210> SEQ ID NO 26
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<212> TYPE: DNA
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<222> LOCATION: D16S753

<400> SEQUENCE: 26

gcagtgccgc ctatttttgt gaat 24

<210> SEQ ID NO 27
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<222> LOCATION: D17S1299

<400> SEQUENCE: 27

accctgatga gatagcactt gagc 24

<210> SEQ ID NO 28
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<212> TYPE: DNA
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<222> LOCATION: D17S1299

<400> SEQUENCE: 28

cactgtgtgg aggtgtagca gaga 24

<210> SEQ ID NO 29
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<212> TYPE: DNA
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<222> LOCATION: D16S539

<400> SEQUENCE: 29

ggggggtctaa gagcttgtaa aaag 24

<210> SEQ ID NO 30
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<212> TYPE: DNA
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<222> LOCATION: D16S539

<400> SEQUENCE: 30

tgtgcatctg taagcatgta tctatc 26

<210> SEQ ID NO 31
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<222> LOCATION: D22S683

<400> SEQUENCE: 31

cgaaggttgc attgagccaa gat 23

<210> SEQ ID NO 32
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<222> LOCATION: D22S683

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ggtggaaatg cctcatgtag aaa 23

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<222> LOCATION: HUMCSF1PO

<400> SEQUENCE: 33

aacctgagtc tgccaaggac tagc 24

<210> SEQ ID NO 34
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<222> LOCATION: HUMCSF1PO

<400> SEQUENCE: 34

ttccacacac cactggccat cttc 24

<210> SEQ ID NO 35
<211> LENGTH: 24
<212> TYPE: DNA
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<222> LOCATION: HUMTPOX

<400> SEQUENCE: 35

actggcacag aacaggcact tagg 24

<210> SEQ ID NO 36
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<400> SEQUENCE: 36

ggaggaactg ggaaccacac aggt 24

<210> SEQ ID NO 37
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<222> LOCATION: HUMTHO1

<400> SEQUENCE: 37

attcaaaggg tatctgggct ctgg 24

<210> SEQ ID NO 38
<211> LENGTH: 24
<212> TYPE: DNA
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<222> LOCATION: HUMTHO1

<400> SEQUENCE: 38

gtgggctgaa aagctcccga ttat 24

<210> SEQ ID NO 39
<211> LENGTH: 29
<212> TYPE: DNA
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<400> SEQUENCE: 39

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<210> SEQ ID NO 40
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<212> TYPE: DNA
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<222> LOCATION: HUMvWFA31

<400> SEQUENCE: 40

ggacagatga taaatacata ggatggatgg 30

<210> SEQ ID NO 41
<211> LENGTH: 24
<212> TYPE: DNA
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<222> LOCATION: HUMF13A01

<400> SEQUENCE: 41

gaggttgcac tccagccttt gcaa 24

<210> SEQ ID NO 42
<211> LENGTH: 24
<212> TYPE: DNA
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<222> LOCATION: HUMF13A01

<400> SEQUENCE: 42

ttcctgaatc atcccagagc caca 24

<210> SEQ ID NO 43
<211> LENGTH: 23
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<222> LOCATION: HUMFESFPS

<400> SEQUENCE: 43

gctgttaatt catgtaggga agg 23

<210> SEQ ID NO 44
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<212> TYPE: DNA
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<222> LOCATION: HUMFESFPS

<400> SEQUENCE: 44

gtagtcccag ctacttggct actc 24

<210> SEQ ID NO 45
<211> LENGTH: 20
<212> TYPE: DNA
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<222> LOCATION: HUMBFXIII

<400> SEQUENCE: 45

tgaggtggtg tactaccata 20

<210> SEQ ID NO 46
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<212> TYPE: DNA
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<222> LOCATION: HUMBFXIII

<400> SEQUENCE: 46

gatcatgcca ttgcactcta 20

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<210> SEQ ID NO 47
<211> LENGTH: 24
<212> TYPE: DNA
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<222> LOCATION: HUMLIPOL

<400> SEQUENCE: 47

ctgaccaagg atagtgggat atag 24

<210> SEQ ID NO 48
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMLIPOL

<400> SEQUENCE: 48

ggtaactgag cgagactgtg tct 23

<210> SEQ ID NO 49
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<212> TYPE: DNA
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<222> LOCATION: D3S1539

<400> SEQUENCE: 49

ccacccttc agcaccag 18

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D19S253

<400> SEQUENCE: 50

atagacagac agacggactg 20

<210> SEQ ID NO 51
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D19S253

<400> SEQUENCE: 51

gggagtggag attacccct 19

<210> SEQ ID NO 52
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D20S481

<400> SEQUENCE: 52

aaagctctct gaagcaggtg t 21

<210> SEQ ID NO 53
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D20S481

<400> SEQUENCE: 53

cagattgcac tagaaagaga ggaa 24

<210> SEQ ID NO 54
<211> LENGTH: 23

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<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D10S1239

<400> SEQUENCE: 54

caccctgtcc ccagctatct gga 23

<210> SEQ ID NO 55
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D9S930

<400> SEQUENCE: 55

agttgaatct tgagtctctc agagtca 27

<210> SEQ ID NO 56
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D4S2368

<400> SEQUENCE: 56

tgtactcatt ttcccgcaat gatg 24

<210> SEQ ID NO 57
<211> LENGTH: 24
<212> TYPE: DNA
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<222> LOCATION: D4S2368

<400> SEQUENCE: 57

tcagaaagta gggtctgggc tctt 24

<210> SEQ ID NO 58
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D16S539

<400> SEQUENCE: 58

tgtgcatctg taagcatgta tctatcat 28

<210> SEQ ID NO 59
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMvWFA31

<400> SEQUENCE: 59

gaaagcccta gtggatgata agaataatca gt 32

<210> SEQ ID NO 60
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMvWFA31

<400> SEQUENCE: 60

ggacagatga taaatacata ggatggatgg ata 33

<210> SEQ ID NO 61
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

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<222> LOCATION: D9S930

<400> SEQUENCE: 61

gctatgggaa ttacaagcag gaaac 25

<210> SEQ ID NO 62
<211> LENGTH: 20
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<213> ORGANISM: Homo sapien
<222> LOCATION: D18S51

<400> SEQUENCE: 62

ttcttgagcc cagaaggtta 20

<210> SEQ ID NO 63
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D18S51

<400> SEQUENCE: 63

ctaccagcaa caacacaaat aaac 24

<210> SEQ ID NO 64
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D21S11

<400> SEQUENCE: 64

atatgtgagt caattcccca ag 22

<210> SEQ ID NO 65
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D21S11

<400> SEQUENCE: 65

tgtattagtc aatgttctcc agagac 26

<210> SEQ ID NO 66
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMTH01

<400> SEQUENCE: 66

gtgattccca ttggcctgtt c 21

<210> SEQ ID NO 67
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMTH01

<400> SEQUENCE: 67

attcctgtgg gctgaaaagc tc 22

<210> SEQ ID NO 68
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D3S1358

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<400> SEQUENCE: 68

gcagtccaat ctgggtgac 19

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
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<222> LOCATION: D3S1358

<400> SEQUENCE: 69

atgaaatcaa cagaggcttg 20

<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMFIBRA

<400> SEQUENCE: 70

ggctgcaggg cataacatta 20

<210> SEQ ID NO 71
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMFIBRA

<400> SEQUENCE: 71

tctatgactt tgcgcttcag ga 22

<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMTPOX

<400> SEQUENCE: 72

gcacagaaca ggcacttagg 20

<210> SEQ ID NO 73
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMTPOX

<400> SEQUENCE: 73

cgctcaaacg tgaggttg 18

<210> SEQ ID NO 74
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D8S1179

<400> SEQUENCE: 74

attgcaactt atatgtattt ttgtatttca tg 32

<210> SEQ ID NO 75
<211> LENGTH: 28
<212> TYPE: DNA
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<222> LOCATION: D8S1179

<400> SEQUENCE: 75

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accaaattgt gttcatgagt atagtttc 28

<210> SEQ ID NO 76
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<222> LOCATION: HUMvWFA31

<400> SEQUENCE: 76

gccctagtgg atgataagaa taatcagtat gtg 33

<210> SEQ ID NO 77
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMCSF1PO

<400> SEQUENCE: 77

ccggaggtaa aggtgtctta aagt 24

<210> SEQ ID NO 78
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMCSF1PO

<400> SEQUENCE: 78

atttcctgtg tcagaccctg tt 22

<210> SEQ ID NO 79
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D16S539

<400> SEQUENCE: 79

gtttgtgtgt gcatctgtaa gcatgtatc 29

<210> SEQ ID NO 80
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D7S820

<400> SEQUENCE: 80

atgttggtca ggctgactat g 21

<210> SEQ ID NO 81
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D7S820

<400> SEQUENCE: 81

gattccacat ttatcctcat tgac 24

<210> SEQ ID NO 82
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D13S317

<400> SEQUENCE: 82

attacagaag tctgggatgt ggagga 26

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<210> SEQ ID NO 83
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<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D13S317

<400> SEQUENCE: 83

ggcagcccaa aaagacaga 19

<210> SEQ ID NO 84
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D5S818

<400> SEQUENCE: 84

ggtgattttc ctctttggta tcc 23

<210> SEQ ID NO 85
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D5S818

<400> SEQUENCE: 85

agccacagtt tacaacattt gtatct 26

<210> SEQ ID NO 86
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: Amelogenin

<400> SEQUENCE: 86

ccctgggctc tgtaaagaa 19

<210> SEQ ID NO 87
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: Amelogenin

<400> SEQUENCE: 87

atcagagctt aaactgggaa gctg 24

<210> SEQ ID NO 88
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: G475

<400> SEQUENCE: 88

taccaacatg aaagggtacc aata 24

<210> SEQ ID NO 89
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: G475

<400> SEQUENCE: 89

tgggttatta attgagaaaa ctcctta 27

<210> SEQ ID NO 90

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<211> LENGTH: 20
<212> TYPE: DNA
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<222> LOCATION: S159

<400> SEQUENCE: 90

gccatgatca caccactaca 20

<210> SEQ ID NO 91
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: S159

<400> SEQUENCE: 91

ccgattttta attgggttgt cttatt 26

<210> SEQ ID NO 92
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: S159

<400> SEQUENCE: 92

gccatgatca caccactaca ctc 23

<210> SEQ ID NO 93
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: S159

<400> SEQUENCE: 93

ccgattttta attgggttgt ctta 24

<210> SEQ ID NO 94
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: G475

<400> SEQUENCE: 94

tgggttatta attgagaaaa ctccttacaa ttt 33

<210> SEQ ID NO 95
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: S159

<400> SEQUENCE: 95

gcacagtggc taattgtacc tt 22

<210> SEQ ID NO 96
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: S159

<400> SEQUENCE: 96

tttttaatag gtcatgattt tgtgat 26

<210> SEQ ID NO 97
<211> LENGTH: 24
<212> TYPE: DNA

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<213> ORGANISM: Homo sapien
<222> LOCATION: D16S539

<400> SEQUENCE: 97

gtttgtgtgt gcatctgtaa gcat 24

<210> SEQ ID NO 98
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMCSF1PO

<400> SEQUENCE: 98

cctgtgtcag accctgttct aagt 24

<210> SEQ ID NO 99
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: C221

<400> SEQUENCE: 99

aacagggata tgcactggta ataga 25

<210> SEQ ID NO 100
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: C221

<400> SEQUENCE: 100

tgcataaaac cctggttggt c 21

<210> SEQ ID NO 101
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D18S51

<400> SEQUENCE: 101

caaacccgac taccagcaac 20

<210> SEQ ID NO 102
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D18S51

<400> SEQUENCE: 102

gagccatgtt catgccactg 20

<210> SEQ ID NO 103
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMTHO1

<400> SEQUENCE: 103

gtgattccca ttggcctgtt cctc 24

<210> SEQ ID NO 104
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D8S1179

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<400> SEQUENCE: 104

tggcaactta tatgtattttt gtatttc 28

<210> SEQ ID NO 105
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: Amelogenin

<400> SEQUENCE: 105

ccctgggctc tgtaaagaat agtg 24

<210> SEQ ID NO 106
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: D3S1358

<400> SEQUENCE: 106

actgcagtcc aatctgggt 19

<210> SEQ ID NO 107
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<222> LOCATION: HUMFIBRA

<400> SEQUENCE: 107

ctatgacttt gcgcttcagg a 21

<210> SEQ ID NO 108
<211> LENGTH: 1000
<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<220> FEATURE:
<222> LOCATION: Clone S159
<223> OTHER INFORMATION: from a pGem3Zf(+)plasmid library

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ggcctgtaat cccacctata atcccaccta ctcgggaggc tgaggcagga 100

gaatcgcttg aacccaggat ggggcgattg cagtgagccg agatcgtgcc 150

actgcactcc agcctgggtg acagagcgag actccatctc aaaaaaaaaa 200

aaaaaaaaca gaatcatagg ccaggcacag tggctaattg taccttggga 250

ggctgagacg ggaggatcga gaccatcctg ggcaccatag tgagacccca 300

tctctacaaa aaaaaaaaaa aatttttttt aaatagccag gcatggtgag 350

gctgaagtag gatcacttga gcctggaagg tcgaagctga agtgagccat 400

gatcacacca ctacactcca gcctaggtga cagagcaaga caccatctca 450

agaaagaaaa aaaagaaaga aaagaaaaga aaagaaaaga aaagaaaaga 500

aaagaaaaga aaaaacgaag gggaaaaaaa gagaatcata aacataaatg 550

taaaatttct caaaaaaatc gttatgacca taggttaggc aaatatttct 600

tagatatcac aaaatcatga cctattaaaa aataataata aagtaagttt 650

catcaaaact taaaagttct actcttcaaa agatacctta taaagaaagt 700

aaaaagacac gccacaggct aagagaaagt acttctaatc acatatctaa 750

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aaaaggactt gtgtccagat taaagaattc ttacacatca ataagacaac          800

ccaattaaaa atcggcaaaa gatttgaaga gatatttaac caaagaaaac          850

atataaatgt gtccgggcgc gatggtaatc ccagcacttt gagaggccga          900

ggcaggcgga tcacttgagg tcaggagttt aggaccagtc tggccaacat          950

ggtgaaaccc tgtctctaat aaaaatacaa aaattagctg ggtgtggtgg         1000


<210> SEQ ID NO 109
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<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<220> FEATURE:
<222> LOCATION: Clone C221
<223> OTHER INFORMATION: from a pGem3Zf(+)plasmid library

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gatgacccct gccctaccca cttgtcatgg cattggggac atgaacacac          100

tttgcacctg tcaggcaagg cttaaacagg gatatgcact ggtaatagaa          150

aagagggact aagttttgtt ttgttttgtt ttgttttgtt ttgttttgtt          200

ttgttttgtt ttgttttgtt ttgtttttct gaagaagtcc ctagaagcgc          250

tcagtgttgg aatgctctct tgtagcagtg gcggctgctg ctggttccgg          300

gtcagatgcc ggaattgggg gtgcgcttgg gtgcagctgc atttcatctg          350

gtcctgggcc tcggtcctgg cttggagagg tgcagctcac agccacttca          400

tggctgggat c                                                   411


<210> SEQ ID NO 110
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<212> TYPE: DNA
<213> ORGANISM: Homo sapien
<220> FEATURE:
<222> LOCATION: Clone G475
<223> OTHER INFORMATION: from a pGem3Zf(+)plasmid library

<400> SEQUENCE: 110

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aagaaaagaa aattgtaagg agttttctca attaataacc caaataagag          150

aattctttcc atgtatcaat catgatacta agcactttac acacatgtat          200

gttatgtaat cattatatca tgcatgcaag gtaatgagta ttattttcct          250

cattttataa aagaggaaac tgatgtttga ggctactttg cttaagaccg          300

cagaactagc aaaggaaaag agaagtgaat gtatc                          335
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What is claimed is:

1. A method of simultaneously determining the alleles present in a set of short tandem repeat loci from one or more DNA samples, comprising:

(a) obtaining at least one DNA sample to be analyzed,

(b) selecting a set of loci of the DNA sample, comprising D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31,

(c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

2. The method of claim **1**, wherein at least four of the short tandem repeat loci are selected from the group consisting of: D5S818, D7S820, D13S317, D16S539, D18S51, D21S11, D3S1358, D8S1179, HUMFIBRA, HUMCSF1PO, HUMTPOX, HUMTH01, and HUMvWFA31.

3. The method of claim 1, wherein at least one of the short tandem repeat loci in the set of loci selected in step (b) is a pentanucleotide tandem repeat locus.

4. The method of claim 1, wherein the set of loci selected in step (b) further comprises a locus which can be used to identify the gender of at least one source of the DNA provided in step (a).

5. The method of claim 2, wherein the multiplex amplification reaction is done using at least one oligonucleotide primer having a sequence selected from at least one of each of the groups of primers consisting of:

SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:80 and SEQ ID NO:81, when one of the loci in the set is D7S820;

SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:82, and SEQ ID NO:83, when one of the loci in the set is D13S317;

SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:84, and SEQ ID NO:85, when one of the loci in the set is D5S818;

SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, SEQ ID NO:79, and SEQ ID NO:97, when one of the loci in the set is D16S539;

SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:77, SEQ ID NO:78, and SEQ ID NO:98, when one of the loci in the set is HUMCSF1PO;

SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:72 and SEQ ID NO:73, when one of the loci in the set is HUMTPOX;

SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:103, when one of the loci in the set is HUMTH01;

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:59, SEQ ID NO:60, and SEQ ID NO:76 when one of the loci in the set is HUMvWFA31;

SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:101, and SEQ ID NO:102, when one of the loci in the set is D18S51;

SEQ ID NO:64 and SEQ ID NO:65, when one of the loci in the set is D21S11;

SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:106, when one of the loci in the set is D3S1358;

SEQ ID NO:70, SEQ ID NO:71, and SEQ ID NO:107, when one of the loci in the set is HUMFIBRA; and

SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:104, when one of the loci in the set is D8S1179.

6. The method of claim 1, wherein the amplified alleles are separated prior to evaluating in step (d), using a separation means selected from the group consisting of polyacrylamide gel electrophoresis and capillary gel electrophoresis.

7. The method of claim 1, wherein the multiplex amplification reaction is done using pairs of oligonucleotide primers flanking the loci analyzed.

8. The method of claim 7, wherein the set of loci is co-amplified using a polymerase chain reaction.

9. The method of claim 7, wherein each of the loci co-amplified in the multiplex reaction in step (b) is co-amplified using a pair of primers which flank the locus, wherein at least one primer of each pair has a fluorescent label covalently attached thereto.

10. The method of claim 9, wherein at least three of the labeled primers have different fluorescent labels covalently attached thereto.

11. The method of claim 1 wherein the at least one DNA sample to be analyzed is prepared from human tissue, wherein the human tissue is selected from the group of human tissue consisting of blood, semen, vaginal cells, hair, saliva, urine, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

12. The method of claim 1, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

13. A method of simultaneously identifying the alleles present in a set of loci of from one or more DNA samples, comprising:

(a) obtaining at least one DNA sample to be analyzed;

(b) selecting a set of loci of the DNA sample, comprising short tandem repeat loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31;

(c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

14. The method of claim 13, wherein the multiplex amplification reaction is done using at least one primer for at least one locus in the set of loci selected in step (b), wherein the primer has a sequence selected from each of the groups of primer sequences consisting of:

SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:101, and SEQ ID NO:102, when one of the loci in the set is D18S51;

SEQ ID NO:64 and SEQ ID NO:65, for the locus D21S11;

SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:66, SEQ ID NO:67, and SEQ ID NO:103, for the locus HUMTH01;

SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:106, for the locus D3S1358;

SEQ ID NO:70, SEQ ID NO:71, and SEQ ID NO:107, for the locus HUMFIBRA;

SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:72, and SEQ ID NO:73, for the locus HUMTPOX;

SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:104, for the locus D8S1179;

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:59, SEQ ID NO:60, and SEQ ID NO:76, for the locus HUMvWFA31;

SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:77, SEQ ID NO:78, and SEQ ID NO:98, for the locus HUMCSF1PO;

SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:58, SEQ ID NO:79, and SEQ ID NO:97, for the locus D16S539;

SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:80, and SEQ ID NO:81, for the locus D7S820;

SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:82, and SEQ ID NO:83, for the locus D13S317; and

SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:84, and SEQ ID NO:85, for the locus D5S818.

15. The method of claim 13, wherein the multiplex amplification reaction is a polymerase chain reaction.

16. The method of claim 13, wherein the amplified alleles are evaluated by comparing the amplified alleles to a size standard, wherein the size standard is selected from the group of size standards consisting of a DNA marker and a locus-specific allelic ladder.

17. The method of claim **13** wherein the at least one DNA sample to be analyzed is prepared from human tissue, wherein the human tissue is selected from the group of human tissue consisting of blood, semen, vaginal cells, hair, saliva, urine, bone, buccal sample, amniotic fluid containing placental cells or fetal cells, and mixtures of any of the tissues listed above.

18. A kit for simultaneously analyzing a set of loci of genomic DNA comprising oligonucleotide primers for co-amplifying a set of loci of the genomic DNA to be analyzed, wherein the set of loci comprises short tandem repeat loci which can be co-amplified, the primers are in one or more containers, the genomic DNA is human genomic DNA, and the loci comprise D3S1358, D5S818, D7S820, D8S1179, D1S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, AND HUMvWFA31.

19. The kit of claim **18**, wherein all of the oligonucleotide primers in the kit are in one container.

20. The kit of claim **18**, wherein at least one of the primers for co-amplifying a locus in the set of loci has a sequence selected from one of the groups of primer sequences consisting of:

SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:101, and SEQ ID NO:102, for D18S51;

SEQ ID NO:64 and SEQ ID NO:65, for D21S11,

SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:38, and SEQ ID NO:103, for HUMTH01

SEQ ID NO:68, SEQ ID NO:69, and SEQ ID NO:106, for D3S1358

SEQ ID NO:70, SEQ ID NO:71, and SEQ ID NO:107,for HUMFIBRA

SEQ ID NO:72 and SEQ ID NO:73, for HUMTPOX

SEQ ID NO:74, SEQ ID NO:75, and SEQ ID NO:104, for D8S1179

SEQ ID NO:76 and SEQ ID NO:40, for HUMvWFA31

SEQ ID NO:77, SEQ ID NO:78, and SEQ ID NO:98, for HUMCSF1PO

SEQ ID NO:29, SEQ ID NO:79, and SEQ ID NO:97, for D16S539

SEQ ID NO:80 and SEQ ID NO:81, for D7S820

SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:82, and SEQ ID NO:83, for D13S317

SEQ ID NO:84 and SEQ ID NO:85, for D5S818.

21. The kit of claim **18**, further comprising reagents for at least one multiplex amplification reaction.

22. The kit of claim **18**, further comprising a container having an allelic ladder.

23. The kit of claim **22**, wherein each rung of the allelic ladder and at least one oligonucleotide primer for each of the loci in the set each have a fluorescent label covalently attached thereto, and at least two of the oligonucleotide primers have a different fluorescent label covalently attached thereto than other primers in the container.

24. A method of simultaneously determining the alleles present in a set of loci from one or more DNA samples, comprising:

(a) obtaining at least one DNA sample to be analyzed;

(b) selecting a set of loci of the at least one DNA sample, comprising at least thirteen short tandem repeat loci which can be co-amplified, wherein at least four of the at least thirteen short tandem repeat loci are selected from the group comprising: D5S818, D7S820, D13S317, D16S539, D18S51, D21S11, D3S1358, D8S1179, HUMFIBRA, HUMCSF1PO, HUMTPOX, HUMTH01, and HUMvWFA31;

(c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and

(d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample.

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